# THE EUSPORANGIATAE

## THE COMPARATIVE MORPHOLOGY
## OF THE OPHIOGLOSSACEAE
## AND MARATTIACEAE

BY

### DOUGLAS HOUGHTON CAMPBELL
PROFESSOR OF BOTANY, LELAND STANFORD JUNIOR UNIVERSITY

# PREFACE.

The great importance of the eusporangiate ferns as the nearest existing relatives of the Paleozoic ancestors of the higher types of flowering plants invests them with an especial interest for the student of plant evolution; and no apology is necessary for presenting at length a summary of our present knowledge of the structure and development of these important plants.

For more than twenty years the writer has been much interested in the study of the Eusporangiatæ and during this time has published a number of works dealing with them. He has had rather unusual opportunities for collecting these forms, and an extensive and representative collection of materials comprising a good many species has been accumulated, so that the time seemed ripe for a comparative study of the group, for the purpose of determining, as far as might be, the relationships existing between the different genera, as well as for throwing some light upon the question of their position in the great series of ferns.

The present memoir is an attempt to present the results of these studies, based mainly upon the writer's own materials, but supplemented by a careful study of the work of other investigators who have described the structure and development of the Eusporangiatæ.

The writer's grateful acknowledgments are due to a number of colleagues who have rendered assistance in various ways. Especially is he indebted to Prof. E. C. Jeffrey, through whose kindness a large number of admirably preserved prothallia and young plants of *Botrychium virginianum* were sent the writer, and in addition a number of valuable slides of the same. Without this material the work on *Botrychium* would have been very incomplete.

To my colleague in Stanford University, Prof. L. L. Burlingame, thanks are due for valuable assistance in the preparation of the photographic plates, as well as for the use of a number of important slides of *Ophioglossum* and *Helminthostachys*.

To Prof. J. C. Willis, of the Botanic Gardens at Peradeniya, the writer would express his appreciation of many kindnesses and assistance in collecting during his stay in Ceylon.

It was the good fortune of the writer to enjoy the unequaled facilities for collecting material offered by the great gardens at Buitenzorg and Tjibodas in Java, where, through the interest and courtesy of the distinguished director, Professor M. Treub, whose recent death was such an irreparable loss to science, means were afforded for securing the most valuable materials used in the preparation of the present work.

<div align="right">Douglas Houghton Campbell.</div>

Stanford University, *April*, 1910.

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## THE COMPARATIVE MORPHOLOGY

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# INTRODUCTION.

It is pretty well agreed among botanists that most of the seed plants, perhaps all of them, are descendants of some fern-like Paleozoic ancestors. The geological record is remarkably perfect in many respects, and our knowledge of many of these Paleozoic fossils is extraordinarily complete. Among the most important contributions made of late years to our knowledge of these Paleozoic fossils is the fact that many of these Paleozoic "ferns" were really seed-bearing plants intermediate in character between the true ferns and the more highly developed flowering plants.

At best the fossil record is very incomplete in regard to many extremely important structural details, and therefore it is especially necessary that these points should be thoroughly studied in such of the existing ferns as, for any reason, seem to be at all closely related to the ancient Paleozoic types.

The name Eusporangiatæ was proposed by Goebel to include the two very peculiar families of fern-like plants, the Marattiaceæ and Ophioglossaceæ, which differ in several important respects from the much more numerous and specialized Leptosporangiatæ, the predominant ferns of the present day. The Eusporangiatæ comprise 100 or more species of widely distributed ferns, of which the Marattiaceæ are mainly tropical in their distribution, while the Ophioglossaceæ include a good many species of temperate regions as well. The Eusporangiatæ are distinguished primarily by the character of the sporangium, which is always much more massive than in the typical ferns, the Leptosporangiatæ. In the latter, the sporangium can almost always be traced back to a single mother cell which usually arises from the surface of the leaf, while in the Eusporangiatæ the sporangium is already multicellular when it is first recognizable.

The Marattiaceæ are in general appearance much like the typical ferns, and there is no question of their relationship to the Leptosporangiatæ. The resemblances are less obvious in the case of the Ophioglossaceæ, and some students of the ferns have expressed the opinion that the Ophioglossaceæ should be separated entirely from the ferns and placed in a distinct class. (See Bower **9.**) A careful comparative study, however, of the two families included in the Eusporangiatæ shows so many close correspondences in structure, both of the sporophyte and gametophyte, that their association together is amply justified.

The Marattiaceæ are known to be very ancient forms, unmistakable members of this family occurring abundantly in Paleozoic formations. The Ophioglossaceæ, on the other hand, are very unsatisfactorily represented in a fossil condition, and for this reason doubt has been thrown on their antiquity, although their structures show strong evidences of an extremely primitive character. Certain of the oldest-known fossil ferns, the Botryopterideæ, may possibly prove to be related to the Ophioglossaceæ, but the evidence at present is not entirely conclusive.

In spite of the unsatisfactory nature of the geological evidence, I am nevertheless strongly inclined to believe that the Ophioglossaceæ, on the whole, represent the most ancient type among the living ferns, this conclusion being based upon a very careful study of the structure of both gametophyte and sporophyte.

There are many practical difficulties in the way of studying these interesting ferns, and these difficulties undoubtedly account for the comparatively small number of researches that have been made upon their development. A few species, like *Ophioglossum vulgatum* and several species of *Botrychium*, are widespread in their

range and occur in the temperate regions of Europe and America; but very much the larger number of these, including all of the Marattiaceæ, are tropical or subtropical plants and are very seldom seen in cultivation, hence material for their study must be collected in their natural habitat.

The gametophytes, or sexual plants, are seldom encountered unless very special search is made for them, and they have been but rarely collected; and even where the spores can be made to germinate, the long time necessary for the growth of the gametophyte under artificial conditions, and the difficulties of rearing them to maturity, readily explain the small number of successful attempts to study these plants under artificial conditions.

As the result of several visits to the tropics of both the Old and New Worlds, the writer has collected material of all of the eusporangiate genera, except the recently discovered *Archangiopteris* from southwestern China and *Macroglossum* from Borneo. It has seemed desirable, with this material as a basis, to make a somewhat comprehensive comparative study of the whole group of the Eusporangiatæ. In this study, especial attention will be devoted to the structure and development of the gametophyte and embryo and to the development of the young vegetative organs of the sporophyte. The general anatomy and histology of the mature sporophyte have already been pretty satisfactorily studied and described for most of the genera and a particularly satisfactory account of the development of the sporangium has been given us in the comprehensive memoirs of Professor Bower (Bower **5, 6**), and a detailed investigation of these points has hardly seemed necessary, although, so far as possible, the results of the work of previous investigators have been verified.

The writer has already published various papers upon the development of both the Marattiaceæ and Ophioglossaceæ, and these have been freely drawn upon in the preparation of the present monograph; but these earlier studies have been materially extended by further work upon the species previously studied and investigations have been made upon a number of species which hitherto have not been examined at all, or have been studied only very incompletely. This is particularly true of the genus *Danæa*, which has received comparatively little attention hitherto. An especially fine lot of material of several species of *Danæa* was collected in Jamaica in 1908 and an extended study of the gametophyte and young sporophyte has been made, the complete results of which are now published for the first time. Attention has also been given to the development of the young sporophyte in the other eusporangiates, particularly in the peculiar genus *Kaulfussia*, which, like *Danæa*, has received less attention from the students of these plants than have the genera *Marattia* and *Angiopteris*.

Of late there has been a tendency, especially among students of the fossil ferns, to lay great stress upon the importance of the structure of the vascular skeleton of the ferns in the study of their phylogeny. It is therefore highly important that a careful examination should be made of the origin and development of the vascular system in those forms, i. e., the Eusporangiatæ, which, there is good reason to suppose, are the nearest relations among living plants of the ancient Paleozoic ferns.

The results of the writer's studies on the development of the fibro-vascular bundles of the sporophyte in all of the eusporangiate ferns have led to some rather unexpected conclusions as to the real nature of the vascular system in these ferns, conclusions decidedly at variance with the views generally accepted at present. These results have seemed sufficiently important to warrant a more extended treatment of this subject than was at first contemplated; and this will explain what might otherwise seem to be a disproportionate amount of space devoted to the origin and development of the fibro-vascular system in the young sporophyte.

# PART I. THE OPHIOGLOSSALES.

The Ophioglossales, the "adder-tongue" ferns, include three genera (of which two are practically cosmopolitan) and embrace numerous species, being sometimes further divided into several subgenera; the third genus is a monotypic one, confined to the tropics of the Old World. These three genera are evidently quite closely related among themselves and may without hesitation be referred to a single family, the Ophioglossaceæ. The genus *Ophioglossum* includes several species of the temperate regions of the whole world, and some of the species, like *O. vulgatum*, are very widespread; the tropical species are much more numerous, but the number of these is very uncertain and much confusion exists as to the limits of certain species. The second genus, *Botrychium*, belongs mainly to the temperate regions, only a small number of species occurring in the tropics, and these are principally confined to the cooler mountain regions. The third genus, *Helminthostachys*, with a single described species, *H. zeylanica*, is a not uncommon fern of the lowland forests of the Indo-Malayan region.

The Ophioglossaceæ are for the most part quite glabrous plants, of small or moderate size. The smallest species, like *Botrychium simplex* and some of the smaller species of *Ophioglossum* (e. g., *O. californicum*, *O. bergianum*, and some of the smaller forms of *O. moluccanum*) may be only 5 or 6 centimeters in height. The largest species of *Botrychium* and *Helminthostachys* sometimes attain a height of 50 centimeters or more, with ample, much-dissected leaves, while the long, ribbon-like, pendent leaves of the epiphytic *Ophioglossum pendulum*, the giant of the family, may reach a length of 1.5 meters.

Except for two species of *Ophioglossum* belonging to the sections *Ophioderma* and *Cheiroglossa*, the Ophioglossaceæ are terrestrial plants, usually growing in soil abounding in humus, and the gametophyte in all cases is a subterranean structure quite destitute of chlorophyll.

The stem is a rhizome, which is short and upright in the terrestrial species of *Ophioglossum* and in *Botrychium*, but is dorsiventral in *Helminthostachys* and the epiphytic species *Ophioglossum pendulum*. In most of the species of the temperate regions only a single leaf is developed each year, but there are some exceptions to this rule in *Ophioglossum*, especially in the tropical species, where there is no interruption of the growth.

The leaves are usually ample, and may be quite undivided, as in most species of *Ophioglossum*, or they may be dichotomously divided in *O. palmatum* and some forms of *O. pendulum;* or they may be much dissected, usually in a ternate fashion, in *Botrychium* and *Helminthostachys*. The sporangia are sometimes very large, and are borne upon characteristic spikes, or "sporangiophores," whose morphological nature is a matter of some controversy. The venation of the leaves is reticulate in all species of *Ophioglossum*, but in the other genera it resembles that of the typical ferns.

With the exception of *Ophioglossum (Cheiroglossa) palmatum*, the sporangiophore is normally attached to the adaxial side of the leaf, usually near the junction of the lamina and petiole; but sometimes it is inserted much lower down, or it

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may arise close to the base of the petiole and appear to be quite independent of the sterile leaf segment. The sporangia range in number from about half a dozen, in some of the smaller forms of *Botrychium simplex* and *Ophioglossum*, to many hundred in such large species as *Botrychium virginianum* and *Helminthostachys*. There is a most interesting series of forms connecting the smaller and simpler types with the large and complicated ones. With the increasing complexity of the sporangiophores, there is usually a reduction in the size of the sporangia, which, however, become better differentiated than in the simpler types.

There is no question about the close relationships existing between the different genera of the Ophioglossaceæ, but there is some difference of opinion as to their connection with other Pteridophytes. While they are probably sufficiently distinct to be relegated to a special order, Ophioglossales, their peculiarities hardly warrant separating them entirely from the ferns. As will be seen later, they offer many points of resemblance to the Marattiales, both in regard to the structure of the adult sporophyte and that of the gametophyte or sexual plant, and the early phases of embryonic development. These resemblances are too numerous and too exact to make it at all likely that the two orders are unrelated.

## I. THE GAMETOPHYTE.

The first discovery of the gametophyte of the Ophioglossaceæ was made by Hofmeister (Hofmeister 1) who, in 1854, found the gametophyte of *Botrychium lunaria;* two years later Mettenius (Mettenius 1) described much more fully the prothallium of *Ophioglossum pedunculosum*, which was cultivated in the botanical garden at Leipzig. No further additions were made to the subject until the writer (Campbell 4) succeeded in obtaining the first germinating stages of *O. pendulum*, collected in the Hawaiian Islands, and those of *Botrychium virginianum;* and the older gametophyte of the latter species was also described. In the year 1898 Jeffrey (Jeffrey 1) published a complete account of the gametophyte and embryo of *Botrychium virginianum*. Later contributions to the subject are those of Lang (Lang 1) on the gametophyte of *Ophioglossum pendulum* and of *Helminthostachys;* and the papers of Bruchmann (Bruchmann 1, 2) on the prothallium of *Botrychium lunaria* and *Ophioglossum vulgatum*. In 1905, Lyon (Lyon 1) published a brief account of the embryo of *Botrychium obliquum*.

In 1906 I collected in Ceylon a few prothallia of a species of *Ophioglossum* which may have been *O. reticulatum*, and shortly afterward, during a stay in Java, a number of prothallia of *O. moluccanum* were found, as well as a few belonging to an undetermined species associated with the latter. During my stay in Java I was fortunate enough to obtain also a large number of prothallia of *O. pendulum*. An account of these has already been published (Campbell 8).

All species of the Ophioglossaceæ that have yet been examined agree in the underground habit and saprophytic nature of the gametophyte, which always has associated with it a peculiar endophytic fungus, or "mycorrhiza," which is undoubtedly connected with the assimilation of organic food. Mettenius states that chlorophyll may be developed if the prothallium appears above ground, and Bruchmann found that this was also true in *O. vulgatum;* but as a rule the prothallium remains subterranean and quite destitute of chlorophyll.

The spores of the Ophioglossaceæ are always of the tetrahedral type, and when ripe possess a moderately thick sculptured outer membrane, which is usually colorless or pale yellow, so that the masses of spores are either white or a pale sulphur-yellow tint. The ripe spore as a rule is packed with granular matter, which makes the

contents appear opaque and obscures more or less the centrally placed nucleus. A marked exception to the ordinary type of spore was that found in an undetermined species of *Ophioglossum* collected at Buitenzorg in Java. This was supposed to be *O. moluccanum*, with which it was growing, but a comparison with typical specimens of the latter species showed marked differences, the most striking being the spores, which were larger than in the type, had much less dense contents, and were especially notable in that they had regularly two nuclei, a condition unique, so far as I know, among the ferns. (See Campbell 8, fig. 157.) The granular contents of the spores include numerous albuminous granules, together with more or less starch and oil.

## GERMINATION IN OPHIOGLOSSUM.

The first successful attempts to germinate the spores of *Ophioglossum* were made by me in 1892, when ripe spores of the epiphytic *O. pendulum* were collected in Hawaii and brought to California. This species was found in Hawaii, growing usually upon the trunks of tree ferns, and the spores were sown upon bits of the bark-like masses of roots, which in the commoner tree ferns of Hawaii (species of *Cibotium*) cover the trunk with a thick, felted mass. These masses of roots were kept in jars, and the spores were sown upon them, a good many of them germinating in course of time. The germination was very slow, the spores often remaining unchanged for months, and none of these young prothallia developed beyond the stage with three cells. This failure to develop further was undoubtedly due to the fact that they did not become infected with the mycorrhiza, which is essential to the full development of the prothallium.

In 1906 ripe spores of the same species were collected in Ceylon and Java. In Ceylon spores were secured at the botanical garden in Peradeniya and in the Barrawa Reserve Forest near Hanwella, where Lang obtained his material. In Java the spores were mostly collected at Tjibodas. In all of these later experiments the spores were sown in humus collected from about the base of the spore-bearing plants. These masses of wet humus were kept in stoppered bottles.

As in the earlier experiments, the germination was slow, the first germinating stages being found only after a month or more, and in some cases the spores remained unchanged for a very long period. The bottles containing the spores sown upon the humus were brought back to California, and a recent examination (September 1909) showed a considerable number of apparently normal spores, as well as living three-celled prothallia, presumably the result of comparatively recent germination.



A. Germinating spore of *Ophioglossum pendulum*. ×360.
B. Optical section of A.
C. Three views of a very young gametophyte of *O. pendulum*, showing infection by mycorrhizal fungus. ×360.

Spores sown in Tjibodas on April 18, 1906, were first found germinating at Buitenzorg on May 24, germination at this time being pretty well advanced. On June 3 a number of these had three cells. The germination (fig. 1) in all cases corresponded closely with the writer's former observations and closely resembles that which we shall see presently in *Ophioglossum moluccanum*. In no case could any chlorophyll be detected,

and apparently the prothallium of *O. pendulum* is strictly saprophytic throughout its existence.

The extremely favorable conditions for plant growth at Buitenzorg made this an unusually promising place for studying germination, and very soon after my arrival there a quantity of plants of *Ophioglossum moluccanum*, or what was supposed to be this species, was secured, and the spores were sown. Subsequent study showed that at least three species grew together at Buitenzorg, so that it is not at all certain that all of the germinating spores and the prothallia which were collected later really belonged to *O. moluccanum*.

Since in all previous experiments with the Ophioglossaceæ germination had been very slow, it was with much surprise that the first lot of spores that were sown, when examined a week later, were found to be germinating very freely and had evidently been growing for some days. New sowings were made, and in some cases the first germination stages were evident within three days from the time the spores were sown. Inasmuch as the spores contain no chlorophyll, this rapid germination is really remarkable. In the most favorable instances the greater part of the spores germinated and many thousand germinating spores were studied.

The first sowings were made upon earth taken from where the plants were growing; the earth was placed in small glass dishes and flooded with water; the spores were then scattered over the surface of the water, some sinking, but the greater part floating on the surface. In later experiments cavities were hollowed out in the earth and these were filled with water, while the rest of the earth was left wet, but not flooded. It was found that the spores germinated more promptly in the water than on the wet earth, and this suggested that probably under natural conditions germination occurs where the spores fall in slight depressions which are filled with water for a time after heavy rains. The prothallia of *Helminthostachys*, to judge from the locality where they were found in the Barrawa Forest, occurred only where the forest was subject to inundation, and it may be that immersion in water is necessary for the germination of *Ophioglossum moluccanum*, or at any rate facilitates germination. The older prothallia of this species which were found in Buitenzorg were growing in low ground between the projecting roots of trees, where water might very well stand for some days in wet weather.

The first sign of germination consists in the enlargement of the spore contents, which soon burst the rigid outer membrane along the three lines upon its ventral face, and through the cleft thus formed the spore contents, surrounded by the colorless inner membrane ("endospore" or "intine") protrude as a blunt papilla (plate 1, fig. 2). The first division wall is transverse and is soon followed by a second wall in the upper cell (i. e., the one turned away from the opening). The second wall is at right angles to the first, and the young prothallium now consists of three cells, the basal one, which is partly exposed through the cleft at the upper side of the spore, and the two upper cells, which have more dense contents than the basal one. The basal cell in position corresponds to the first rhizoid in the germinating spore of the typical ferns, but it was never found to become extended into a true rhizoid, and no rhizoids were seen in any of the very young prothallia.

While *Ophioglossum moluccanum* and *O. pendulum* agree very closely in the early stages of germination, in the latter species no trace of chlorophyll can be detected at any time, but in *O. moluccanum* it is not uncommon to find from one to three chloroplasts present. These chloroplasts are very pale in color, but it is certain that a small amount of chlorophyll is present in some cases.

While most of the young prothallia of *Ophioglossum moluccanum* do not advance beyond a three-celled stage, a few were found in which there were four cells, but all

attempts to carry them beyond this stage failed and after exhausting the spore contents they soon died. The small amount of chlorophyll occasionally found in these young prothallia is evidently insufficient for their independent growth, and after a few weeks the granular contents all disappear and the cells soon collapse, showing that the young gametophyte has died from starvation.

A number of specimens of the rare *Ophioglossum intermedium* were collected near Buitenzorg and a few ripe spores were also secured. These spores contain somewhat less dense contents than those of the other species described and have a more delicate epispore. Spores were sown on March 30 in Buitenzorg, and when examined about two weeks later no germinations were found, nor did a second examination about the end of April show any further results. On May 21, however, two three-celled prothallia were found, and subsequently a small number of others, but no later stages were discovered. The young prothallia appeared in every respect like the similar stages in the other species (plate 1, fig. 6).

In *Ophioglossum pendulum* a number of young prothallia were found which had increased very much in size and undergone further division. The first of these were observed on April 3, and had developed from spores sown in Peradeniya on February 9. These young prothallia (fig. 1, *c*) had from four to six cells. It was found that in each case the mycorrhiza had connected itself with the young prothallium, and evidently had caused a stimulus in its growth. In every case where the young prothallium had more than three cells there was found associated with it the mycorrhiza, which could be easily seen to penetrate into the basal cell. The infection was in all cases due to fragments of mycelium, and in no case to anything which could be interpreted as spores. The fungus was apparently growing free in the humus where the spores were sown. This soil, as we shall see, was taken from about the roots of the sporophytes which furnished the spores.

The free surface of the basal cell has its wall decidedly thickened, and it was here that the infection took place in all the specimens seen. The branching mycelium of the mycorrhiza was closely applied to the surface of the cell and a haustorium was sent down through the cell wall into the basal cell (fig. 1, *m*). This haustorium is pointed at first, but after it penetrates into the cell it enlarges and assumes the form of a somewhat thickened worm-shaped body, much thicker than the free mycelium outside. In the cell infected with the fungus, the contents show the peculiar aggregated appearance characteristic of the infected cells of the older prothallia. On the 6th of April a specimen with seven cells was found. About a month later, a number of other specimens were observed also, some of these having as many as thirteen cells (plate 1, fig. 9). This was the largest number found in any of the young prothallia.

The divisions of these young prothallia are mainly in the lower cells, so that the apex, as in the prothallium of the true ferns, develops mainly from the lower of the two original prothallial cells. The basal cell, however, also undergoes divisions, and there is no very marked difference between the lower and upper ends of the prothallium. At this stage there is a marked resemblance, except for the absence of chlorophyll, to the early germination stages of *Lycopodium cernuum* (Treub 1). The mycorrhiza penetrates the cells adjacent to the one first infected, but leaves the apical region free, and this region probably remains permanently free from the endophyte, as it does in the adult prothallium.

The number of young prothallia found was too small to make it possible to determine exactly what may be considered to be the normal succession of cell division, and whether at this early stage there is a definite apical cell could not be decided. As will be seen from the figures, there is evidently a good deal of variation in the

early divisions. In these larger prothallia there is already the beginning of an axial tissue. Whether the cell x (plate 1, fig 9) is to be regarded as an apical cell, it would be hard to say.

Owing to my departure from Java about three months after the first observations were made, it was impossible to trace the development of the prothallia further, but this much is certain—without the infection of the fungus, growth will not proceed beyond the three-celled stage, and apparently in *O. pendulum* no chlorophyll will develop under any conditions, and the prothallium from its earliest stages must be considered saprophytic in its nutrition. Whether the oval body described as the product of germination is to be considered as a sort of tubercle, such as is found in *Lycopodium cernuum*, must be decided by further investigations. Lang's descriptions and figures of the smallest specimens which he discovered would indicate that this is not the case in *O. pendulum;* but the tuberous body usually found at the base of the older prothallia in *O. moluccanum* (and this is true also in *O. vulgatum*) would indicate that in these species it is not impossible that a primary tubercle is first formed and subsequently the fertile branch.

## THE ADULT GAMETOPHYTE OF OPHIOGLOSSUM.

In 1856 Mettenius (Mettenius 1) found the gametophyte of *O. pedunculosum* Desv., a tropical species, growing spontaneously in the pots where the plants had been cultivated in the botanical garden at Leipzig. He did not succeed, however, in making the spores germinate. These prothallia were slender, subterranean bodies, sometimes branched, sometimes without branches. They ranged in length from 1.5 lines to 2 inches (fig. 3, *A, B*). There was usually present a basal enlargement or tuber, from which the fertile part of the prothallium extended. The older portions were brownish in color; the growing tips of the branch white. From the surface there grew numerous short brown rhizoids. Archegonia and antheridia grew more or less intermingled and were formed in large numbers. Except for the greater size, these prothallia closely resemble those of *O. moluccanum* collected by me in Buitenzorg; and as *O. pedunculosum* Desv. has been held to be a synonym of *O. moluccanum* Schlecht, it is possible that Mettenius's plants were the same as those found by me growing in Buitenzorg in Java.*

The next account of the prothallium of *Ophioglossum* is that of Lang (Lang 1). He collected in Ceylon specimens of the prothallia of *Ophioglossum pendulum.* These were found in the Barrawa Reserve Forest, not far from Colombo, and were buried in the humus accumulated between the leaf bases of *Polypodium quercifolium,* an epiphytic fern to which *O. pendulum* is often attached. I visited this same locality in February, 1906, but was unsuccessful in collecting the prothallia, although I obtained numbers of the sporophytes. Some time after, however, when in Java, I found a very large number of prothallia which were growing in much the same way as those collected by Lang, except that in this case the fern to which the *Ophioglossum* was attached was the widespread bird's-nest fern, *Asplenium nidus.*

Bruchmann has given a detailed account of the prothallium of the widespread *O. vulgatum,* which agrees closely in its essential details with *O. pedunculosum* and *O. moluccanum.* Bruchmann's specimens were collected in the Thuringian Forest, in a depression that was subjected at times to overflow, a condition paralleled by the locations where the prothallia of *Helminthostachys* were collected by Lang and

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* Christensen in his recent Index Filicum (1906) regards *O. moluccanum* as a synonym of *O. pedunculosum.* Through the kindness of Professor K. Goebel, I recently had an opportunity of examining some specimens of *O. pedunculosum* growing in the botanical garden in Munich. These plants were the descendants of the specimens in Leipzig described by Mettenius and certainly very closely resembled the typical *O. moluccanum* from Buitenzorg.

myself, and also like the locality where most of my specimens of *O. moluccanum* were found in Java. This, in connection with my experiments in germinating the spores of *O. moluccanum*, makes it not unlikely that actual submersion is a necessary condition for the germination in the terrestrial species of *Ophioglossum*.

In April 1906, about fifty prothallia of *O. moluccanum* were collected by me at Buitenzorg, where, as we have seen, this species is extremely abundant. Only a small number of these were young enough to show the young reproductive organs, and most of them had already developed the young sporophyte. The greater part of these prothallia were found growing together in a slight depression between the projecting roots of a tree.

These prothallia were slender bodies, from 5 to 10 millimeters in length, and none of them branched. They showed a more or less conspicuous basal tuber, like that described by Mettenius for *O. pedunculosum*, and indeed they very closely resembled his figures of the simpler forms of that species, but are very much smaller. The youngest specimen found (plate 1, fig. 10) consisted of a small, irregular tuberous body of a brownish color, from which grew a short appendage or branch, the tip of which was white. The older ones also showed this basal tuber, but the cylindrical branch was much longer. Owing to their slender form, the prothallia are not always easily distinguishable from roots, and in some cases a microscopic examination is necessary before one can be sure of their real nature. Growing from the surface are scattered short brown hairs like those described by Mettenius for *O. pedunculosum*. Archegonia and antheridia are formed at an early period, and can be traced to the base of the fertile branch, or in some cases may be found even upon the tuber. In these specimens the reproductive organs seemed to be formed in rather smaller numbers than is the case either in *O. pedunculosum* or *O. vulgatum*. Among the prothallia was one very much larger than the others which had very large numbers of old archegonia. It is highly probable that this represents a second species, but unfortunately there was no means of determining to which of the two or three forms associated under the name *O. moluccanum* it belonged.

The cells of the tuber and the lower part of the branch contain the characteristic endophytic fungus, but the greater part of the fertile branch is quite free from this, and the cells appear almost transparent, but they contain numerous small starch granules. The endophyte is much more abundant in the cells of the tuber. The hyphæ, which stain readily with gentian violet, are irregular in outline and branch freely. Very often branches can be seen piercing the walls of adjacent cells.

In the living condition the pointed apex of the prothallium is pure white, and even with a hand lens the projection of the antheridia is clearly evident. Mettenius noticed this "varicose" appearance of the smaller prothallia in *O. pedunculosum*. A median section of the branch shows that the end is decidedly pointed and has a clearly defined apical cell. Owing to the very small amount of material available, no satisfactory transverse section of the apex could be made, and it can not be told whether the apical cell in transverse section is three-sided, as described by Bruchmann for *O. vulgatum*, or is four-sided, as it is in *O. pendulum*. In the smaller specimens antheridia were more numerous than archegonia, although several of the latter were present. The antheridia arise, in general, in acropetal succession, but it is not unlikely that secondary ones may be formed also. The archegonia are scattered among the antheridia apparently without any definite order. In some specimens, especially in the larger ones, the old archegonia were found in great numbers, many more than the antheridia. In other specimens a considerable part of the prothallium was quite destitute of either archegonia or antheridia, and in this respect the prothallium of *O. moluccanum* differs from that of *O. pedunculosum* or *O. vulgatum*.

The gametophyte of *O. moluccanum* shows a greater or less number of rhizoids both at the base and along the fertile branch (plate 1, fig. 10). In the more slender forms, however, these rhizoids are few. They are in some cases two-celled, but more commonly consist of a single elongated cell. It is not unusual to find within this the penetrating filament of the mycorrhiza, as has been described for other species of *Ophioglossum*. The rhizoids are much longer relatively than in *O. pendulum*. According to Bruchmann, the rhizoids are quite absent from the prothallia of *O. vulgatum*.

Mettenius states that in *O. pedunculosum* the prothallia often appear above the surface of the earth, and they then become somewhat flattened and sometimes divided into several small lobes, and in such cases chlorophyll is developed. Mettenius, however, does not note any further development of these green lobes. Bruchmann found that chlorophyll might also develop in *O. vulgatum*, when the prothallia were exposed to the light, but he did not find any flattening of the apex. Owing to the very small number of growing prothallia found by me, I could not test the power of developing chlorophyll in *O. moluccanum*, but the occurrence of chromatophores in the germinating spores makes it highly probable that chlorophyll may be developed in the older prothallia under the stimulus of light.



Fig. 2.

Gametophytes, *pr*, and young sporophytes of *Ophioglossum moluccanum* and allied species, slightly enlarged. C and F from Hakgala, Ceylon; the others from Buitenzorg, Java; *k*, bud on primary root; *l*, primary leaf; *l²*, secondary leaf; *t*, tuberous enlargement at base of gametophyte.

The small size of the prothallium in *O. moluccanum* and the cessation of growth after the sporophyte is formed indicate that the gametophyte lives only for one season, and this is probably the case also in *Helminthostachys*. In this respect *O. moluccanum* and its allies differ markedly from *O. pendulum* and *O. vulgatum*, where the gametophyte lives for many years.

At Hakgala, in Ceylon, an undetermined species of *Ophioglossum* of the type of *O. reticulatum* is common. After careful search, a few prothallia which closely resembled those of *O. moluccanum* were collected (fig. 2, *C*, *F*). The material was too scanty to make a detailed study possible, but from the external appearance it is likely that the structural details would closely resemble those of *O. moluccanum*.

The prothallium of *Ophioglossum vulgatum*, according to Bruchmann (Bruchmann 1), closely resembles that of *O. moluccanum*, but is very much larger, sometimes reaching a length of 6 centimeters, and it is not infrequently branched

(fig 3, *C*). There is a more or less conspicuous basal tuber, like that found in *O. moluccanum*, and from this extends a branch bearing the reproductive organs;

but, unlike the prothallium of *O. moluccanum*, there seems to be a complete absence of rhizoids. In general, the form is more irregular than that of *O. moluccanum* and moreover it is very long-lived. The branches may become detached and thus form new individuals. From the rate of growth in specimens kept under observation for several months, Bruchmann concluded that they



FIG. 3.

A, B. Gametophytes of *Ophioglossum pedunculosum* (after Mettenius).
C, D. Gametophytes of *O. vulgatum* (after Bruchmann).
E-G. Gametophytes of *O. pendulum*. *sp*, young sporophyte; *r*, secondary root.

might live for twenty years or more. Where the apical growth of a branch is interfered with, there may be a formation of adventitious buds, a phenomenon which is also common in *O. pendulum*.

*Ophioglossum (Ophioderma) pendulum*, a remarkable epiphytic species wide-spread through the tropics of the Old World and reaching to Hawaii, is the giant of the order, the pendent ribbon-shaped leaves sometimes attaining a length of 1.5 meters. The sporophyte grows rooted in masses of humus, either upon the rough trunk of a tree fern or palm, or hanging from the mass of humus accumulated about the base of certain epiphytic ferns. The bird's-nest fern, *Asplenium nidus*, furnishes the favorite substratum for this species in the forest of Tjibodas in Java, where my material was collected.

The young prothallium in *O. pendulum* is an ovoid body, the somewhat smaller forward portion corresponding to the fertile branch in *O. moluccanum* (see Lang **I**, page 25). The older ones become very much larger than in any other species that has been described, and they branch freely in all directions, except as their growth is controlled by their position. They are always found buried in a mass of humus between the imbricated leaf bases of the fern, and are often much flattened by the pressure of the leaves between which they are confined. Branches extend in all directions, but their growth is to some extent controlled by the inclosing leaf bases and also by the tangled mass of roots of the fern, which grow in all directions through the mass of humus and among which the branches of the prothallium of the *Ophioglossum* extend. In one instance several hundred were collected from one large plant of *Asplenium nidus*. The prothallia closely resemble Lang's figures and descriptions, but in many cases are very much larger and more extensively branched than any of the specimens collected by him in Ceylon (plate 1, figs. 11–14). The larger prothallia may be stellate in form, but they are usually very irregular. The branches penetrate in all directions between the dense tangle of roots which the *Asplenium* sends into the humus between its persistent leaf bases, and on pulling these back a mass of fine humus is found, held together by the mat of roots so that it can be removed intact. The prothallia are excessively brittle, and it is practically impossible to remove the larger ones without a loss of some of the numerous branches.
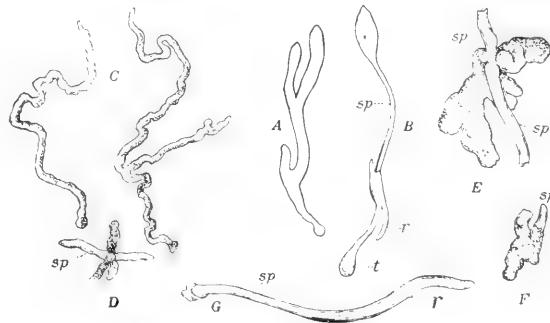
These break off at the least touch, and no doubt serve to propagate the gametophyte, which is apparently capable of unlimited growth in this way. It is often impossible to say whether the smaller gametophytes that are found loose in the humus are anything more than branches which have become spontaneously separated from the larger prothallia.

The older parts of the gametophyte are dark brown in color, but the tips of the branches are white, as in the other species. The branching, as we have seen, may be very irregular, and old fragments kept moist often send out great numbers of adventitious buds (plate 1, fig. 11) which apparently in time develop into normal prothallia. A number of the commoner forms are shown in the figures. Plate 1, fig. 14, represents the largest one met with. This is by no means complete, as a
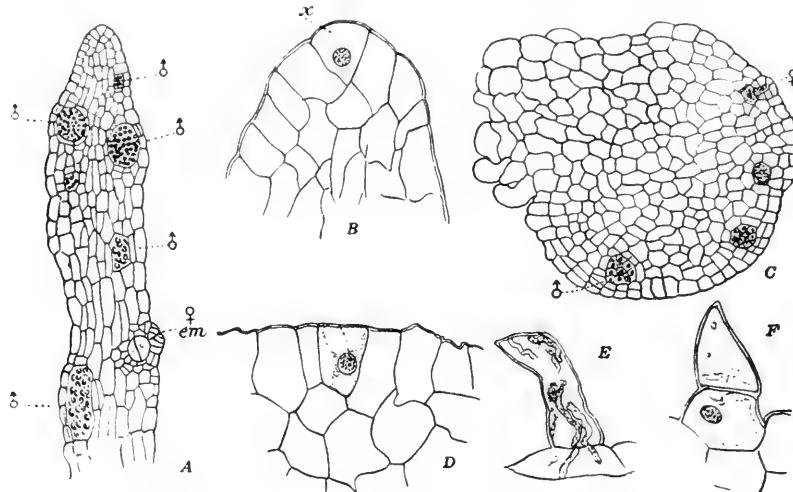


Fig. 4.

A. Longitudinal section of apex of gametophyte of *Ophioglossum moluccanum*. ♂ antheridia; ♀ archegonia; *em*, two-celled embryo.
B. Apex more highly magnified, showing apical cell, *x*.
C. Transverse section of a branch of the gametophyte of *Ophioglossum pendulum*.
D. Apical cell of same.
E, F. Short hairs from gametophyte of *O. pendulum*; in E may be seen the endophytic fungus filaments.

number of branches were unavoidably broken off in removing it from among the tangle of roots in which it was embedded. This specimen measured about 15 millimeters in diameter—more than twice the size of the largest specimen secured by Lang. The surface of the older parts of the prothallium shows a slightly roughened appearance due to the numerous very short papillate hairs which occur upon it. These are never of the slender form found in *O. moluccanum* and perhaps are not properly to be considered as rhizoids. Dotted over the surface are pale brown spots, easily seen with the naked eye, and these on examination are found to be the large, empty antheridia. The branching is sometimes dichotomous, but lateral branches may arise at almost any point, and old fragments of the prothallia, as already indicated, often develop many adventitious buds, a condition of things which apparently obtains also in the long-lived prothallia of *O. vulgatum*. The rate of growth of prothallia kept by the writer for more than a year, as well as their

position in the humus about the plant, where they are found in the older parts between leaves which must have been dead for many years, indicate that they are very long-lived and, as we have seen, by the ready separation of the branches they are easily propagated.

<div align="center">THE HISTOLOGY OF THE GAMETOPHYTE.</div>

The younger portions of the growing branches are composed of thin-walled, colorless parenchyma, whose cells have a conspicuous nucleus, and usually numerous small starch granules. In the older portions of the gametophyte the tissue becomes infected with the often-described endophytic fungus. Practically all of the cells of the basal tuber are thus infested, but in the fertile branches the central tissue is usually free from the fungus, and this medulla-like central tissue is surrounded by a more or less definite mantle of cells, in which the endophyte is especially luxuriant. The outermost cells are practically free from fungus, although new infections probably may take place there through these outer cells. In *O. moluccanum* the endophyte is much less developed than in *O. pendulum*, this no doubt being correlated with the much briefer duration of the gametophyte in the former species. According to Bruchmann, the endophyte is strongly developed also in *O. vulgatum*. The endophyte, as in the other forms that have been studied, is quite absent from the apical region of the branches. The limits of the infested zone are not very clearly marked and any cell of the older tissue of the gametophyte may harbor the fungus.
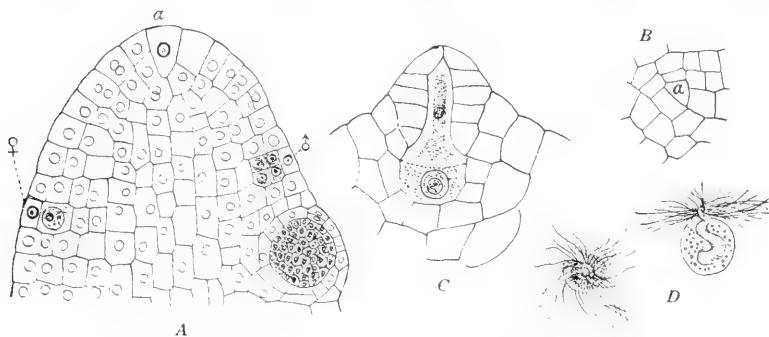


FIG. 5.

A. Longitudinal section of gametophyte apex of *Ophioglossum vulgatum.* ×150.
B. Transverse section of gametophyte apex. *a*, the apical cell.
C. Archegonium. ×225.
D. Two free spermatozoids. ×550. (All figures after Bruchmann.)

The apical cell in *O. pendulum* is usually a four-sided pyramid, and not tetrahedral, as it is in *O. vulgatum*. In longitudinal section (fig. 4, *D*), the apical cell appears triangular with a fairly regular segmentation, but there is also active division in the adjacent tissue and apparently the segmentation of the apical cell is not very rapid. Cross-sections show the apical cell to be approximately four-sided (fig. 16, *A*), but the sides are not always of equal length and sometimes it is almost triangular in outline; possibly it may be that in some cases, as in *O. vulgatum*, it is tetrahedral. There seems to be no absolute rule, however, as to the succession of divisions in the young segments. A more or less definite superficial layer arises from the first periclinal divisions, but anticlinals follow rapidly.

### THE GAMETOPHYTE OF BOTRYCHIUM.

Hofmeister (Hofmeister 1), in his studies of the Archegoniates, has included *Botrychium lunaria*, whose prothallium he discovered in 1854. No further contributions to our knowledge of the subject are recorded until 1893, when the writer discovered at Grosse Ile, Michigan, a number of old prothallia of *B. virginianum*, with the young sporophytes attached. The earliest germination stages of this species were also secured. In 1895, Prof. E. C. Jeffrey collected at several points in Canada a large number of prothallia of this species, and his account (Jeffrey 1) is much the most satisfactory one which had appeared up to that time. In the spring of 1903 Lyon (Lyon 1) discovered prothallia of *B. obliquum* in Minnesota, and the following year secured a few specimens of *B. simplex* and *B. matricariæfolium*, but he has only published a brief note in regard to these species. He states that the reproductive organs in *B. obliquum* "differ essentially from those of *B. virginianum*," but he does not explain in what these differences consist. Bruchmann, who has added so much to our knowledge of the gametophyte of the European Lycopodiaceæ and Ophioglossaceæ, has recently given a very satisfactory account of the prothallium and embryo of *B. lunaria*, which he found in various parts of Germany and Switzerland. He corrected certain errors made by Hofmeister in his account of the same species. (Bruchmann 2.)
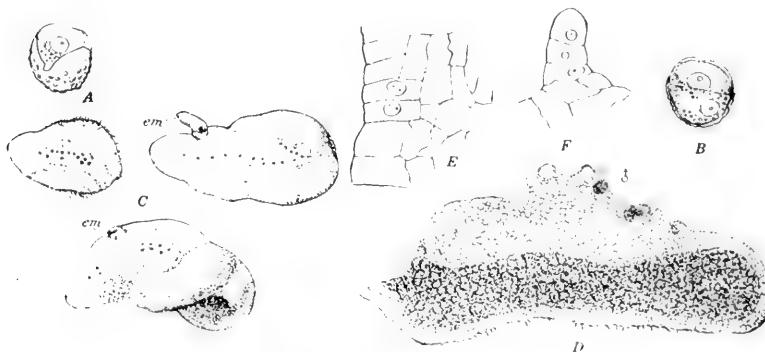


Fig. 6.—*Botrychium virginianum.*

A, B. Germinating spore (B, optical section).
C. Three gametophytes, ×3; *em*, embryo.
D. Section of gametophyte, ×12; the shaded region is that occupied by the endophyte.  ♂ antheridia.
E. Apical region of gametophyte, ×150.
F. Short multicellular hair or paraphysis.

Owing to the kindness of Professor Jeffrey, a large number of prothallia and young sporophytes of *B. virginianum*, together with several slides showing the sexual organs and embryos, were put at my disposal. This has made it possible for me to make a very satisfactory study of the reproductive organs and embryo in this species.

The spores of *Botrychium*, like those of *Ophioglossum*, are of the tetrahedral type and are quite colorless, their contents showing the usual granular appearance, but without any trace of chlorophyll. The early stages of germination are exactly like those of *Ophioglossum*, the first division wall being transverse, this being then followed by a second wall in the inner cell at right angles to the primary wall (fig. 6, *A*, *B*). A few chloroplasts were seen in some of the cells, but this does not seem to be a constant character and perhaps was an abnormality due to the spores having

germinated in the light. Bruchmann (Bruchmann **2**, page 207) also found the earliest stages in *B. lunaria*, and these corresponded exactly to those in *B. virginianum*.

The gametophyte of *B. virginianum* is a large, tuber-like body, which grows at a depth of about 10 centimeters below the surface of the earth. The smallest of the specimens found by Jeffrey were 2 millimeters in length by 1.5 millimeters in breadth, exceeding in size the fully developed prothallia of *B. lunaria*. The older ones may reach a length of 20 millimeters. The young prothallia are quite smoothly oval in outline (fig. 6, *C*), while the older ones are more or less



FIG. 7.
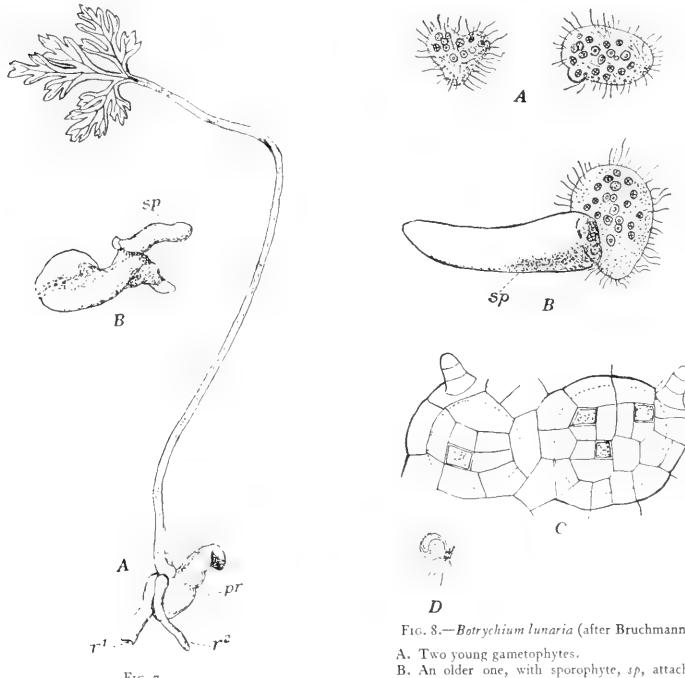
A. Young sporophyte of *Botrychium virginianum* attached to gametophyte, *pr*. ×2.
B. A gametophyte with small sporophyte, *sp*. ×4.

FIG. 8.—*Botrychium lunaria* (after Bruchmann).

A. Two young gametophytes.
B. An older one, with sporophyte, *sp*, attached. ×16.
C. Surface view of two antheridia, showing opercular cells (shaded). ×100.
D. A spermatozoid. ×500.

irregular, and occasionally the anterior end is divided into two equal lobes, probably as a result of dichotomy. As in *Ophioglossum*, the older parts of the gametophyte are brownish in color, while the young apex appears white. In their earlier stages they are covered with numerous rhizoids, which disappear more or less completely as the prothallium becomes older. The forward end is slightly pointed and there is a definite growing point. The antheridia make their appearance first, and together with the archegonia are formed only upon the upper surface of the dorsiventral thallus, which is noticeably different in form from the cylindrical radially constructed prothallium of *Ophioglossum*.

2

The antheridia form a more or less evident row, even in the very young prothallium, and later this median row of antheridia is raised upon an elevated ridge, upon whose sides the archegonia later make their appearance (fig. 6, *D*).

The gametophyte of *B. lunaria* closely resembles the early stages of *B. virginianum*. It is very much smaller and rarely exceeds a length of 2 millimeters, and, like the young stages in *B. virginianum*, it is covered with many long rhizoids (fig. 8, *A*, *B*.) The smaller prothallia are globular or oval in shape; the larger ones somewhat heart-shaped. Hofmeister states that the antheridia occur upon the upper surface and the archegonia below; but Bruchmann found that both archegonia and antheridia were confined to the upper surface, as in *B. virginianum*. As in the latter, also, the dorsal ridge is present, bearing the antheridia upon its crest and the archegonia upon its flanks.

The large prothallia of *B. virginianum* live for many years, and Jeffrey even found a plant bearing spores, which was still connected with a prothallium. It is probable that the very much smaller prothallia of *B. lunaria* have a shorter duration, but in this species also the prothallium persists for some time after the young sporophyte is established.

### THE HISTOLOGY OF THE GAMETOPHYTE OF BOTRYCHIUM.

In *B. virginianum* the forward part of the prothallium is made up of colorless tissue, which extends for some distance beyond the youngest antheridia. The apical meristem in this species (fig. 6, *E*) lies on the upper side of the prothallium; in a vertical longitudinal section it shows a group of columnar cells, one of which is
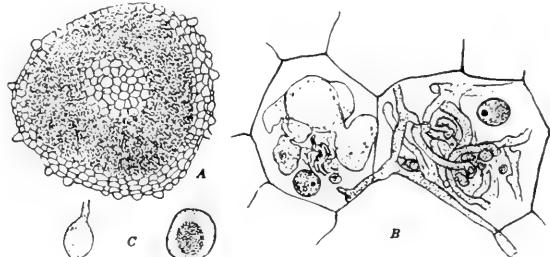


FIG. 9.

A. Transverse section of a gametophyte of *Ophioglossum pendulum*, showing position of endophytic fungus. ×20. The shaded region is occupied by the endophyte.
B. Endophyte from prothallium of *Botrychium virginianum*.
C. Sporangium-like enlargements of endophyte.

probably the apical cell; but this is not absolutely certain. In *B. lunaria* (see Bruchmann 2, page 209) the apical meristem occupies the middle region of the anterior surface of the prothallium, and is of very limited extent. As in *B. virginianum*, its cells appear columnar in form, but neither in longitudinal section nor in a surface view could a single initial cell be recognized.

The rhizoids in *B. virginianum* are from 1 to 4 millimeters in length and may be multicellular, especially those which arise from the crest and flanks of the pro-thallium, while those which originate from the base are unicellular and longer than these multicellular ones. It is not unlikely that these multicellular structures are rather of the nature of paraphyses than true rhizoids. In *B. lunaria* the rhizoids seem to be, usually at least, unicellular. In both species the rhizoids soon turn brown and their walls become strongly cutinized. Both Jeffrey and Bruchmann

observed the penetration of fungus hyphæ into the rhizoids, and they believe that the fungus which occurs within the thallus is, mainly at least, due to this method of infection. From a comparison with the early infection of the young prothallium in *Ophioglossum*, it seems to me more probable that the young prothallium in *Botrychium* also is infected at a very early period and that the endophyte, once established within its tissues, grows with the development of the gametophyte, the secondary infection through the rhizoids of the older gametophyte being of minor importance.

In both species of *Botrychium* the infected region comprises the greater part of the central tissue, leaving only a comparatively narrow peripheral region free from the endophyte. This uninfected area is thicker upon the upper surface and comprises the whole of the meristematic region, together with the developing sexual organs. As in *Ophioglossum*, this uninfected tissue contains small starch granules in considerable numbers, but not much else in the way of granular contents. The invasion of the fungus results in the destruction of the starch and the accumulation of large amounts of oil. This oil, according to Jeffrey, is not readily soluble in alcohol, and the cells containing it, both in fresh and stained sections, appear dark-colored.

## THE GAMETOPHYTE OF HELMINTHOSTACHYS.

The monotypic *Helminthostachys zeylanica* is not uncommon throughout the lowlands of the Indo-Malayan region and often occurs in large numbers. The only account yet published of the gametophyte is that of Lang (Lang 1). The prothallia which he describes were collected in part by himself in the Barrawa Reserve Forest in Ceylon in March 1901. Other material studied by Lang was collected at the same place by Mr. Coomara Swamy. I made a visit to the same locality in February 1906, and also found a considerable number of prothallia, but all of these had been fertilized and had attached to them the young sporophyte, so that no young reproductive organs were found. The forest where they were collected is subject to inundation from a river which runs through it, and it was in the parts that had been overflowed that the young plants were discovered. This makes it not unlikely that, as in the case of *Ophioglossum moluccanum*, germination is favored by having the spores immersed in water, and this may be a necessary condition for the first stages of germination.

A quantity of ripe spores were collected and various attempts were made to germinate these, but without success, and, as none of the specimens obtained by Lang were very young, the early history of the prothallium still remains to be investigated.

As in the other Ophioglossaceæ, the prothallia are subterranean, occurring at a depth of from 5 to 6 centimeters in the earth. In form (figs. 10, 11) they are somewhat intermediate between *Botrychium* and *Ophioglossum*, but are on the whole more like the latter. They are somewhat irregular in outline, with a broad base, recalling the basal tuber of *O. moluccanum*, but this tuber is relatively larger and more lobed. From this basal tuberous portion a short upright branch extends, much as in *Ophioglossum moluccanum*, but it is relatively thicker and shorter. The whole gametophyte is radial in structure, as in *Ophioglossum*, and thus differs strikingly from the dorsiventral gametophyte of *Botrychium*. The basal enlarged portion is brown in color and covered with rhizoids, which are mostly absent from the upper, more slender part upon which the reproductive organs are borne.

Lang found that there is a tendency to diœcism in the prothallia, some producing only antheridia (figure 11, *A*), while in others archegonia predominate,

although in the latter a few antheridia are almost always developed before the arche-
gonia appear.

In the male prothallium the basal "vegetative" portion is relatively small
and often is irregularly lobed, these lobes being not of the nature of true branches, but merely the result of unequal growth. The female gametophyte (fig. 10) has the basal region relatively larger and is even more irregular in form than the male, while the fertile region is shorter and wider. Some of the more elongated types of the male gametophyte are quite similar to the simple prothallium of *Ophioglossum moluccanum*. As in the other genera, the endophytic fungus is always present and occupies much the same position as in *Botrychium*.



Fig. 10.

A. Gametophyte (*pr*) of *Helminthostachys zeylanica*, with young sporophyte attached; *r*, primary root. × 4.
B. An older sporophyte, with fully-developed second leaf. *s.2. cot*, rudimentary cotyledon.
C. Young second leaf, showing venation. × 5.

Lang found that the growth is due to the activity of a single apical cell having the form of a four-sided pyramid, much like that found in *Ophioglossum pendulum*. The lateral segments divide by periclinal walls, and from the superficial cells thus formed originates the layer of tissue from which the sexual organs arise. The inner cells contribute to the axial tissue of the gametophyte. The antheridia in the male plants are evenly distributed about the periphery, so that in cross-section they form a nearly uniform circle. The axial tissue, especially in the male prothallium, has the cells much elongated, and Lang thinks they are useful in the conduction of plastic material from the basal region of the prothallium to the growing point.

The outer part of the basal region consists of two or three layers of somewhat flattened cells, which, like the corresponding tissue in *Botrychium*, are free from the endophyte. From some of these superficial cells there are developed unicellular, elongated rhizoids, with markedly cutinized walls. This cutinization extends to the outer walls of the superficial cells, while the inner walls of these cells, as well as those of the inner tissue, show the cellulose reaction. The formation of the lobes of the basal region are due, not to definite apical growth, but to irregular cell divisions in the outer layers, which remain free from the fungus which occupies the greater part of the central region of the lobes, as well as the axis of the main shoot of the prothallium.

The central tissue is made up of about equal parts of infected and uninfected cells. The latter, as in the other cases investigated, contain starch granules which are absent from the cells harboring the fungus. Fungus hyphæ were seen in many cases to penetrate the rhizoids, but it is highly probable that in *Helminthostachys* also there is a primary infection at an early stage in the germination of the spores.

Lang notes that in the older male prothallia the fungus is dead and the further growth of the prothallium is dependent upon the amount of reserve food (mainly
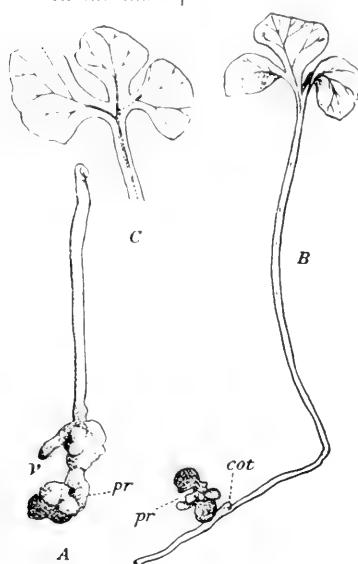
starch) which is left in the cells. It is not clear what rôle the endophyte plays in the manufacture of the reserve food materials upon which the further growth of the prothallium depends. It is highly probable, however, that some of the necessary organic elements are derived from the destruction of the fungus tissues which serve as food for the further development of the prothallium. While no data are at hand to prove this, the lack of permanent growing tissue in the prothallium and the complete destruction of the fungus make it highly probable that the life of the gametophyte in *Helminthostachys* is restricted to a single season, as it is in *Ophioglossum moluccanum.*
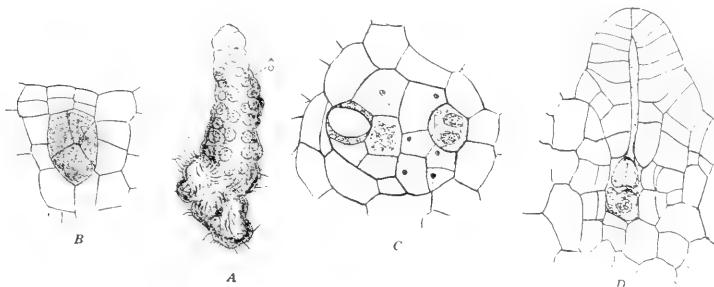


FIG. 11.

A. Male gametophyte of *Helminthostachys*. ×8.   C. Surface view of ripe antheridium, showing opercular cells.
B. Young antheridium.   ×225.   D. An old archegonium.   × about 200.
(Figs. A-C, after Lang.)

## THE ENDOPHYTE.

The endophytic fungus which inhabits the gametophyte in all of the Ophioglosseæ is very much alike in all of the species, differing only slightly in size in different forms (fig. 9). A special study was made of this endophyte in *Ophioglossum pendulum,* where it is especially well developed. As we have already seen, the endophyte is absent from the younger parts of the prothallium, but in the older parts it is exceedingly conspicuous. Sometimes fragments of a fungus are found outside the prothallium, growing in the humus, and these are evidently the same forms that infect the very young prothallium when it arises from the germinating spores. These external hyphæ in some cases have an occasional septum, and this is also the case in those forms which infect the young prothallium. In all of the hyphæ seen within the prothallium, however, these septa seem quite absent. The infection of the prothallium through the rhizoids has been noted in all the species, but, as we have already stated, it is probable that the endophyte is derived mainly from the first infection of the very young gametophyte. An examination of the young cells before the entrance of the fungus shows a conspicuous nucleus and numerous starch grains, which stain very strongly with gentian violet. The invading mycelium, whether from the outside of the prothallium or from the adjacent cells, penetrates the cell wall and ramifies within the cell, the growth being entirely intracellular. The hyphæ are noticeably thicker than those of the external mycorrhiza.

The endophyte is perfectly fixed with 1 per cent chromic acid and it stains well with the double stain of gentian violet and safranine, the walls assuming a violet color and the numerous nuclei staining deep red. In the younger hyphæ, which are of varying sizes, the protoplasm is densely granular, but in the older ones

the granular appearance disappears to a considerable extent, though the nuclei continue to stain strongly. The fungus is quite variable in form. Sometimes the filaments are nearly straight, running from cell to cell and branching sparingly. Sometimes a cell is completely filled with a dense tangle of hyphæ, while in other cases there are sack-like vesicles of very irregular form. Not infrequently, quite regular, nearly globular bodies are seen, recalling the oögonia of the Peronosporeæ. These at first contain comparatively few nuclei, scattered through the granular cytoplasm, but in the older ones the nuclei are very numerous and decidedly larger. It looks sometimes as if this were a preparation for the formation of spores, but no certain evidence of the formation of such spores could be seen, although in several instances there was an appearance which might point to this. Structures resembling the "conidia," described by Jeffrey for *Botrychium* (Jeffrey 1, page 12), were seen and are probably the same thing.

As the fungus invades the young cells, irregular, strongly-staining clumps are formed by the aggregation and breaking down of the starch grains. The nucleus of the host cell appears to be but slightly affected by the growth of the fungus, and can usually be found quite unchanged, even in those cells which are almost completely filled by the endophyte. Finally, the thin-walled vesicular growths of the fungus are quite broken down and probably serve to nourish the cells of the gametophyte, which is thus parasitic upon the cells of the fungus. The systematic position of the fungus is doubtful. The varicose, swollen hyphæ found at certain stages closely resemble a parasitic fungus, *Completoria complens*, which is sometimes extremely destructive to green fern prothallia. The oögonium-like organs, often present, suggest *Pythium*, a parasitic fungus belonging to the Peronosporeæ. Jeffrey (Jeffrey 1, page 13) thinks the endophyte may fairly be regarded as an intermediate form between the two genera, *Completoria* and *Pythium*, and says that in this case *Completoria* should be placed in the Peronosporeæ instead of in the Entomophthoreæ, where it has been placed by some students of the Fungi.

## THE SEXUAL ORGANS.

### THE ANTHERIDIUM

The antheridium in all of the Ophioglossaceæ is of much the same type and closely resembles that of the Marattiaceæ and also *Equisetum* and *Lycopodium*. The mother cell does not project at all above the level of the adjacent tissue, and in all cases the first division separates a superficial or cover cell from an inner one, the latter by further divisions giving rise to a mass of sperm cells which may be very numerous. The cover cell also divides, sometimes only in one plan, sometimes (e. g., in *Botrychium*) having also periclinal divisions. At maturity the antheridium may project more or less strongly, but this is not always the case.

### THE ANTHERIDIUM OF OPHIOGLOSSUM

The antheridium was first described by Mettenius in *Ophioglossum pedunculosum*, and later Lang described it for *O. pendulum* and Bruchmann for *O. vulgatum*. The present account is based mainly upon my own studies of *O. moluccanum* and *O. pendulum*.

The mother cell of the antheridium, which may arise very close to the growing point of the prothallium, lies flush with the neighboring cells, but later it may become more or less elevated above the surface, forming a prominence which, in slender prothallia, like those of *Ophioglossum moluccanum*, may give an irregular undulate outline to the branch which bears them (fig. 4, *A*). In *O. pendulum*

this projection is less marked, although this may be somewhat decided in the ripe antheridium. The first division in the inner cell is usually vertical, but may be transverse, at least in *O. pendulum* (fig. 13, *A*). The second walls intersect the first at right angles, and there are always four nearly equal inner cells resulting (fig. 13, *D*). The third set of walls is vertical, and the next, in some cases at least, is in the same direction. This is not true, however, of the deeper and narrower type of antheridium. Further divisions occur until the number of spermatocytes is very large. The fully developed mass of spermatocytes is plainly visible to the naked eye, and in *O. pendulum* may reach a diameter of more than 325 μ. 250 cells and upward may be counted in a single section of a large antheridium, and this would mean that there are several thousand in the whole antheridium—perhaps more than in any other Pteridophyte. In *O. moluccanum* (fig. 12), the number of spermatocytes is much smaller, while in this regard the antheridium of *O. vulgatum* appears to be intermediate between that of *O. moluccanum* and *O. pendulum*.
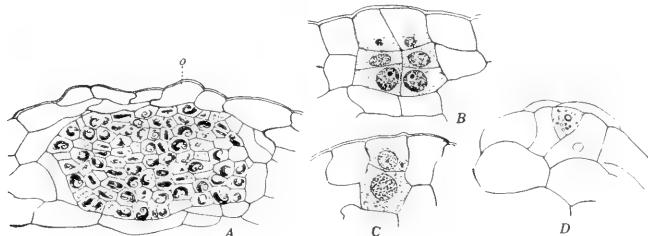


Fig. 12.—*Ophioglossum moluccanum*.
A. Ripe antheridium. ×180.
B, C. Young antheridia. ×320.
D. Surface view of antheridium, showing opercular cell.

The nuclei of the young cells show a conspicuous nucleolus, which becomes less marked in the later stages of the antheridium. According to Mettenius, the outer wall of the antheridium in *O. pedunculosum* is composed of two layers of cells; but both Lang and Bruchmann found that the central part of this outer wall of the
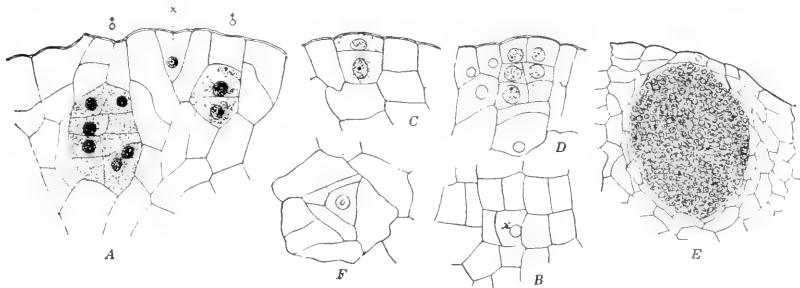


Fig. 13.—Development of the antheridium in *Ophioglossum pendulum*.
A. Median section of gametophyte, apex, showing apical cell, *x*, and two young antheridia, ♂. ×175.
B. Transverse section of apex; *x*, apical cell.
C, D. Median sections of young antheridia.
E. Median section of a nearly ripe antheridium.
F. Surface view of ripe antheridium; *o*, opercular cell.

antheridium is but one cell in thickness, and I have verified this for both of the species under consideration.

The first wall to be formed in the cover cell is a nearly median one and vertical (fig. 12, *B*), and this is followed by a second wall which intersects it, as well as one of the lateral walls of the primary cover cell, so as to include a nearly triangular cell. In this triangular cell there are later formed, as both Bruchmann and Lang showed, a varying number of segments arranged spirally in the fashion of the segments of a three-sided apical cell (fig. 13, *F*). The same thing occurs in *Lycopodium* (Treub I). I have also found a similar condition in the antheridium both of the Marattiaceæ and of *Equisetum*. The last-formed triangular cell is the opercular cell (fig. 13, *F*, *o*). From the prothallial tissue which adjoins the sperm cells are cut off flattened cells which surround the sperm cells with a more or less definite layer of "mantle" cells. The limits of the original cover cell are usually plainly visible in both longitudinal and surface sections.

### THE ANTHERIDIUM OF BOTRYCHIUM.

The antheridia in *Botrychium* occur only upon the dorsal surface of the gametophyte, which is always monœcious. The first antheridia in *B. virginianum* (see Jeffrey I, page 8), form a small cluster which is not noticeably raised above the general level of the prothallium, and from this primary cluster extends a single median line of antheridia toward the apex of the gametophyte. Later this median region becomes raised and forms a conspicuous ridge along whose crest the antheridia are borne.    In *B. lunaria*, according to Bruchmann, the arrangement is much the same.
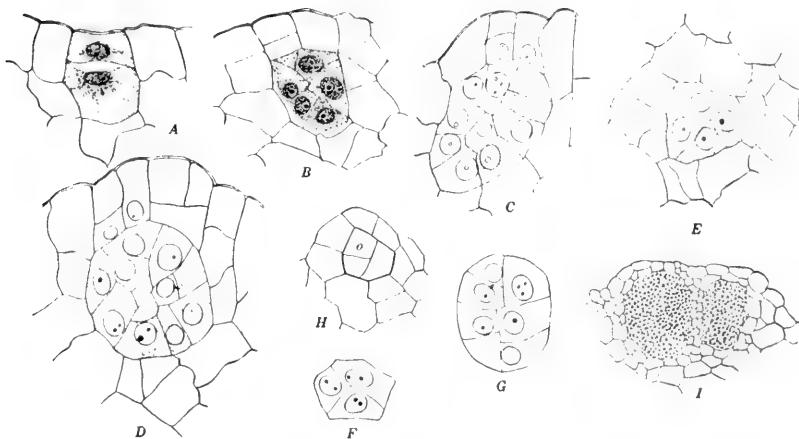


FIG. 14.—Development of the antheridium in *Botrychium virginianum.*

A–D. Longitudinal sections of young antheridia.   × 320.
E–H. Transverse sections.  F, G show only the young spermatocytes.  H, surface view showing opercular
      cell, *o*.   I, section of two ripe antheridia.   × 80.

The development of the antheridium is very much like that of *Ophioglossum*. The first division, as in the latter, is a periclinal one, separating the primary cover cell from the mother cell of the spermatocytes. The divisions in the cover cell, however, differ somewhat from those in *Ophioglossum*, in that periclinal walls

are formed in some of these, so that the cover becomes to some extent double. Jeffrey states that the cover is two layers throughout, but Bruchmann found that some of the cells—two and sometimes three—remain undivided and that one of these undivided cells functions as the opercular cell. I have examined this point carefully in *Botrychium virginianum* and find that there is an opercular cell in this species also. The division walls in the primary cover cell usually intersect at right angles, so that the opercular cell is four-sided, instead of triangular, as in *Ophioglossum* (fig. 14, *H*); but exceptionally it may have the triangular form. In the dehiscence of the antheridium only a single opercular cell is destroyed, not two superimposed cells, as Jeffrey supposed to be the case.

The divisions in the central cell are very much the same as in *Ophioglossum*. Whether the first division is regularly transverse or vertical could not be determined, as no examples of the first division were found. The earliest stages seen after the first separation of the cover cell had four inner cells arranged quadrant-wise, but it was not clear which was the primary wall. The quadrant division is, usually at least, followed by an octant division, and these early divisions may sometimes be recognized for a long while, as there is not much displacement of the cells due to the subsequent divisions. In the later stages the primary divisions can no longer be made out clearly. The number of sperm cells finally formed may be very large— more than a thousand in the largest antheridia—thus nearly or quite equaling the number found in *Ophioglossum pendulum*, although the sperm cells are very much less in size than in the latter and the antheridium correspondingly smaller. Sometimes the antheridium is divided into irregular chambers, apparently due to the persistence of some of the early division walls (fig. 15, *B*).
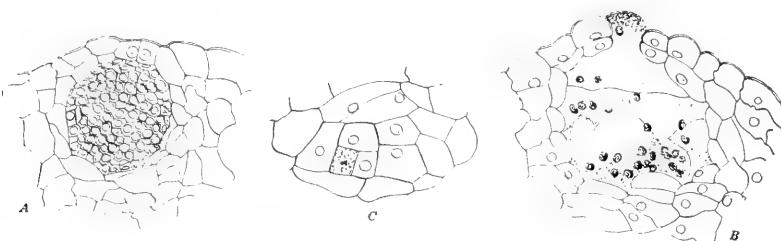


FIG. 15.—*Botrychium virginianum.*

A. Longitudinal section of a nearly ripe, but rather small, antheridium. × about 200.
B. Section of a ripe antheridium which has discharged the spermatozoids; some cell-walls have remained, forming irregular chambers, and retaining some of the spermatozoids.
C. A surface view of a ripe antheridium, showing square opercular cell.

The antheridium of *B. lunaria*, according to Bruchmann, is smaller than that of *B. virginianum*, but the size of the spermatocytes and spermatozoids seems to be about the same (fig. 8, *D*).

### THE ANTHERIDIUM OF HELMINTHOSTACHYS.

The antheridium of *Helminthostachys* has been studied by Lang, and it is evident from his account that it resembles that of *Botrychium* much more than it does that of *Ophioglossum*. As in the former, the primary cover cell undergoes periclinal divisions as well as anticlinal ones, but there are from two to four cells which do not divide by periclinal walls, and one of these cells becomes the opercular cell (fig. 11, *C*). These four undivided cells stain more strongly than the other cells of the cover, but only one of them is broken down at the time of dehiscence. These

cells, as in the case of *Botrychium lunaria*, are nearly square in outline, most of the walls in the cover cells being at right angles to each other, as in *Botrychium*.

The divisions in the central cell are very similar to those in *Ophioglossum* and *Botrychium*, and the original quadrant divisions are perceptible for some time. The number of spermatocytes, as in *Ophioglossum* and *Botrychium*, may be very large. The spermatozoids were not seen by Lang, but from a comparison of the nuclei of the spermatocytes they are apparently of about the same size as those of *Botrychium*.

### SPERMATOGENESIS.

*Ophioglossum* is especially suited to a study of spermatogenesis, owing to the very large size of the spermatozoids. Those of *O. pendulum* are probably the largest known among the Pteridophytes. In material fixed with 1 per cent chromic acid, or weak Flemming's solution, and stained with gentian violet and aniline safranine, the coloring is beautifully clear and the blepharoplast stains with extraordinary sharpness. The spermatozoids of *O. pendulum* are larger than those of *O. moluccanum* or *O. vulgatum*, but the development is very much the same.

If the sperm cells are examined previous to the final division to form the spermatocytes (plate 1, fig. 16), the nucleus will be seen to have a small but distinct nucleolus and a dense reticulum. The whole nucleus stains strongly with safranine. The cytoplasm is fairly dense, with granules of various kinds in it. In material fixed with Flemming's solution there are often small black specks, probably fatty bodies, which sometimes interfere somewhat with the study of the cytoplasm. In well-stained sections the blepharoplasts may be seen as two small, rounded bodies of a violet color (plate 1, fig. 16, *bl*) lying near the nucleus. Several cases of the final nuclear divisions were met with, but all of these were in material fixed with chromic acid, and the blepharoplasts were not very well differentiated (plate 2, fig. 22). The nuclear spindle is very distinct, and the very numerous chromosomes are so crowded that it was quite impossible to determine their number exactly, but it is very large. In the young spermatocytes (plate 2, fig. 26) the nucleus shows a more or less conspicuous reticulum, but the nucleolus has disappeared, as it usually does at this stage in all the forms that have been studied. The further development of the spermatozoid corresponds very closely with that of *Equisetum* (see Campbell 4 and Belajeff 1, 2). One of the blepharoplasts in the primary spermatocyte goes with each daughter cell (the definitive spermatocyte) and can be seen as a distinct rounded body lying near the nucleus (plate 2, fig. 24). In some cases what appeared to be the blepharoplast was lying in a depression at the periphery of the nucleus, and looked very much like a nucleolus.

Before the nucleus undergoes any marked change, the blepharoplast begins to elongate (plate 2, figs. 26, 27) and assumes the form of a pointed, slender, strongly staining body lying near the nucleus. This body is really somewhat ribbon-shaped, and more pointed at one end than the other, and it is also somewhat curved, even in its earlier stages. A transverse section appears crescent-shaped. The nucleus now elongates slightly and the reticulate structure becomes very conspicuous (plate 2, fig. 25). There are large, strongly stained granules, which are probably composed of several more or less coherent chromosomes, as the number of these granules is very much less than the number of chromosomes in the nuclear plate of the dividing nucleus. The blepharoplast continues to elongate, and in favorable cases the young cilia can be seen growing from it, but none of my preparations showed the cilia nearly so plainly as Belajeff's figures show them in *Equisetum* and *Gymnogramme*. There is no doubt, however, that the cilia arise in much the same way as Belajeff describes. The nucleus now becomes indented on one side and assumes a crescent

shape, elongating and also becoming more or less flattened. One end becomes narrower and sharply pointed, the other remaining thicker and rounded. The reticulum at this time stains with great intensity and shows a tendency to coalesce, which in the final stage results in an almost homogeneous, deeply-staining mass, composed apparently of the completely fused chromosomes. In successful preparations the nucleus at this stage stains a clear carmine red, in strong contrast to the bright violet of the blepharoplast. With the complete coalescence of the chromosomes the volume of the nucleus is noticeably decreased (plate 1, fig. 19). The blepharoplast forms a spirally coiled narrow band, from which the cilia can be seen to grow, following its curve, but the blepharoplast is not in close contact with the nucleus.

The spermatocytes and the nuclei are rather smaller in *Ophioglossum moluccanum* than in *O. pendulum*. In the older stages of the spermatozoid, the nucleus in the former species is decidedly more elongated and more sharply pointed at both ends (plate 1, fig. 18). In this respect it more nearly resembles the spermatozoids of *Botrychium* and those of the true ferns; while in the larger, comparatively short nucleus, as well as in some other respects, the spermatozoid of *O. pendulum* is strikingly like that of *Equisetum*. The number of cilia is large, but the exact number could not be determined.

Surrounding the spermatozoid and included in its coils is a considerable amount of cytoplasm. I was not successful in obtaining any living spermatozoids, although many attempts were made to do this, but in a number of sections of the opened antheridium some were found in which the spermatozoids had been retained. While these were usually more or less distorted, some were very well fixed and gave a good idea of the structure of the free spermatozoid (plate 2, fig. 28). The cilia were very much distorted in some cases, but in some they were clearly seen. There is one thick posterior coil mainly composed of the nucleus, which is very much larger than that of the sperm cell before it is discharged from the antheridium. The nucleus has the form of a slightly coiled thick band, tapering somewhat at both ends, but more markedly so in front. Beyond this extends a second coil, composed, apparently, mainly of cytoplasm. This second coil extends into a third much smaller one, which seems to be a flattened band along whose upper edge the blepharoplast is closely applied (plate 2, fig. 28). The resemblance of the spermatozoid to that of *Equisetum* is very strong, but the nucleus is even more shortened than in the latter.

The spermatozoid of *Ophioglossum pendulum* is larger than that of any Pteridophyte that has yet been described. The cytoplasmic envelope and vesicle are not very clearly separated and probably are similar to those of the free spermatozoids of *O. vulgatum* or of *Equisetum*. Sometimes this protoplasmic envelope completely surrounds the lower part of the spermatozoid and reminds one somewhat of the peculiar spermatozoids of the Cycads. Owing to their large size, the spermatozoids were often sectioned, and in some of the sections the blepharoplast was free from the body of the spermatozoid and the attachment of the cilia was very easily seen (plate 2, fig. 29, *a*).

Mettenius figures the free spermatozoids of *O. pedunculosum*, but his figures are certainly not accurate. Bruchmann, who has figured those of *O. vulgatum* (fig. 5, *D*), shows that they closely resemble the spermatozoids of the true ferns, but are more massive, and the vesicle which envelops the posterior coils adheres more closely to the spermatozoid than is usual in the ferns. In these respects it resembles the spermatozoid of *O. pendulum*.

Jeffrey examined the development of the spermatozoids in *Botrychium virginianum*, which closely resemble those of *Ophioglossum*. Owing to the method of

staining which he employed, the blepharoplast was not clearly evident. I have examined the developing spermatozoids in this species, using the material which Professor Jeffrey was so kind as to send me, but employing the double stain of safranine and gentian violet, which was used in the study of *Ophioglossum*. While the differentiation was inferior to that of *Ophioglossum*, where the material was fixed with Flemming's solution, nevertheless the presence of the blepharoplast was unmistakable.

The spermatocytes are only about half as large as those of *Ophioglossum pendulum*, and the spermatozoids correspondingly smaller; hence less favorable for the study of details. It is evident, however, that the changes in the nucleus and blepharoplast are essentially the same as in *Ophioglossum* (plate 2, figs. 34 to 37). The nucleus, however, becomes much more elongated and the spermatozoids are more slender, in both of which respects it shows a resemblance to the spermatozoids of the typical ferns.

The study of the free spermatozoids was confined to those which happened to have become fixed within the open antheridium and within the venter of recently opened archegonia. These were in some cases very satisfactorily fixed and stained, and showed very well indeed the general form of the spermatozoid. The cilia, however, were not as a rule very well fixed, and while they are evidently numerous their number could not be made out.

Bruchmann's figures (fig. 8, *D*) of the free spermatozoid of *Botrychium lunaria* agree closely in form and size with those of *B. virginianum*. In both species the vesicle attached to the spermatozoid is more distinct than in *Ophioglossum*, and in this respect also the spermatozoid shows a likeness to the true ferns.

### THE ARCHEGONIUM.

The archegonium in the Ophioglossaceæ is very much like that of the typical ferns in its general development. The mother cell of the archegonium, like that of the antheridium, is first divided by a periclinal wall into an outer cover cell, which later gives rise to the four rows of neck cells, and an inner cell which, as in the typical ferns, divides again by a transverse wall into a lower or basal cell and a central cell, the latter subsequently giving rise to the egg cell and the canal cells. A marked feature in the archegonium of the Ophioglossaceæ is the inconspicuous character of the ventral canal cell, which very often it is impossible to show, and it is possible that in some cases the ventral canal cell may not be formed at all, although I think this is doubtful. In general the archegonium is much alike in the three genera, but the neck is much shorter in *Ophioglossum* than in *Botrychium* or *Helminthostachys*.

### THE ARCHEGONIUM OF OPHIOGLOSSUM.

My own studies of the development of the archegonium of *Ophioglossum* were based mainly upon a study of *Ophioglossum pendulum*. Only a very small number of the young archegonia was secured in *O. moluccanum*, and so it was impossible to make a complete study in this species. Lang accurately figures several stages of the archegonium in *Ophioglossum pendulum*, and Bruchmann has described quite completely its development in *O. vulgatum*. Bruchmann failed to see the two nuclei of the neck canal cell which Lang correctly figures for *O. pendulum*. These two nuclei are invariably present in both *O. pendulum* and *O. moluccanum*, and it is to be expected that they will also be found in *O. vulgatum*, as they are constantly present also in *Botrychium virginianum* and in all of the ferns that have been accurately examined. Neither Lang nor Bruchmann saw the ventral canal cell, which is exceedingly difficult to demonstrate.

In *Ophioglossum* the young archegonia may be found near the apex of the prothallial branch, but they may also arise at a considerable distance back of it. In general, like the antheridia, they arise in acropetal succession.

The mother cell, like that of the antheridium, is sometimes broad, sometimes narrow and deep, and the cover cell is correspondingly shallow or deep. The first division of the inner cell takes place shortly after the cover cell is cut off. The next division is in the cover cell, which first divides by a vertical wall, a second vertical wall being formed almost immediately, intersecting the first and dividing the cover cell into four practically equal cells, arranged quadrant-wise (fig. 16, *A*). The middle cell next divides by a transverse wall into the primary neck canal cell and the central cell (fig. 16, *F*). The canal cell pushes up between the four primary neck cells, which presently divide by nearly horizontal walls, so that there are two tiers of neck cells. One or both of these divide again later, so that each row of neck cells consists of three or four. Rarely there may be five cells in one or more of the rows.
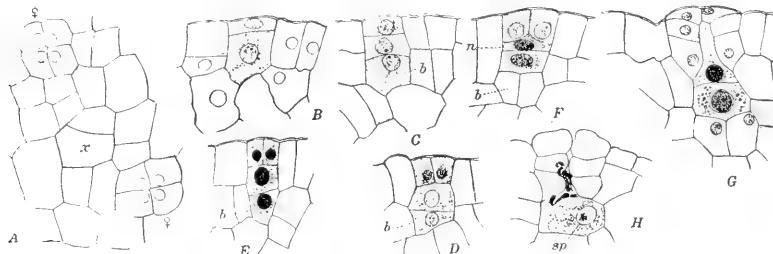


FIG. 16.—Development of the archegonium in *Ophioglossum pendulum*. ×180.

A. Transverse section of gametophyte apex, showing two young archegonia, ♀, and apical cell, *x*.
B–G. Successive stages in the development of the archegonium; seen in longitudinal section; *n*, neck canal cell; *b*, basal cell.
H. Recently fertilized archegonium; *sp*, a spermatozoid within the egg nucleus.

The neck canal cell is very conspicuous, its base being broad, and the upper part narrower and extending to the uppermost neck cells. The large and conspicuous nucleus soon divides into two, but as a rule there is no division wall, although occasionally two distinct neck canal cells may be present. Sometimes both of the nuclei remain in the broad basal part of the cell, and sometimes one is at the base and one nearer the apex. This arrangement seems to depend upon the direction in which the nuclear division takes place.

The basal cell divides by a vertical wall at about the same time that the primary canal cell is cut off from the central cell. The basal cell subsequently undergoes further divisions, but its limits are readily distinguishable up to the time that the archegonium is mature (fig. 16, *G*).

In its earlier stages, the archegonium of *Ophioglossum pendulum* bears a striking resemblance to that of the Marattiaceæ, which the mature archegonium more nearly resembles than it does that of *Botrychium*. According to Bruchmann's account, *O. vulgatum* has a neck somewhat longer, and this is also true of *O. moluccanum*. Even when mature, the neck projects but little above the surface of the prothallium, although there is some elongation of the cells at the time of dehiscence (fig. 16, *G*).

The ventral canal cell is very difficult to demonstrate, and one is sometimes inclined to doubt whether it is formed at all in some cases; neither Lang nor Bruch-

mann was able to detect it in *O. pendulum* and *O. vulgatum*. It is probable that its apparent absence in most of the archegonia is due to the fact that it is formed very late and is extremely inconspicuous. The same apparent absence of a ventral canal cell in the Cycads and some Conifers has been shown, on more critical study of the material, to be due to the small size of the ventral nucleus and to its very evanescent character. In nearly all of the archegonia examined, just before they opened there was present a vesicular body above the egg, which was probably the ventral canal cell much distended with fluid preparatory to the opening of the archegonium. A small nucleus, or what looked like one, could sometimes be seen, but it must be said that its nuclear nature was not above suspicion (plate 2, fig. 33).

Just before the archegonium is ready to open, the egg cell, which up to this time is compressed above by the basal wall of the neck canal cell, becomes distended and pushes up the base of the canal cell, which thus becomes concave below. It is probably about this time that the ventral canal cell is cut off. Unfortunately, no cases were found showing mitosis in the central cell, but there seems no good reason to doubt that a ventral canal cell is, usually at least, cut off.
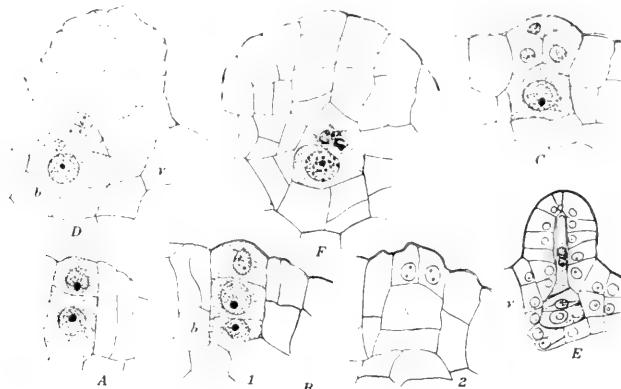


FIG. 17.—Development of the archegonium in *Botrychium virginianum*.

A-D. Longitudinal sections.  ×320.
E. Ripe archegonium, showing ventral canal cell *v*.  ×165.  (After Jeffrey.)
F. Recently fertilized archegonium, showing spermatozoids within venter.  ×320.

The neck canal cell does not show the complete disorganization which is common, but retains its form up to the time that the archegonium opens. With the opening of the neck there is some elongation of the outer neck cells, but there is decidedly less projection above the surface of the prothallium in *O. pendulum* than is the case in *O. vulgatum*. The nucleus of the egg cell is large, but it does not always stain readily, except the nucleoli, and it may be that the same resistance to stains is the reason why its sister nucleus in the ventral canal cell is so difficult to see.

### THE ARCHEGONIUM OF BOTRYCHIUM.

The archegonia in *Botrychium* are found usually upon the flanks of the median ridge, upon whose summit are borne the antheridia. In their early stages they closely resemble those of *Ophioglossum* (fig. 17). As in the latter, there is some variation in the form of the mother cell, which may be quite deep and narrow, or relatively broad and shallow. The subsequent divisions in the central cell and in

the cover cell agree very closely with those in *Ophioglossum;* but the neck cells are more numerous and at maturity the neck projects much more strongly than is the case in *Ophioglossum.* There may be as many as seven or eight cells in each row of the neck, which, except that it is quite straight, resembles that of the typical ferns. It is especially like that of *Osmunda,* in which the neck is also straight. The neck canal cell (fig. 17, *D*) becomes much elongated, and the nucleus divides, as in *Ophioglossum,* but in most cases at least there is no division of the canal cell itself.

As in *Ophioglossum,* the ventral canal cell is very inconspicuous, and often impossible to detect. Jeffrey (fig. 17, *E*) figures a very evident ventral canal cell, but the nucleus is much smaller than that of the egg or neck canal cell. I have also found what seemed to be a ventral canal cell in the archegonium shortly before it opened, but, as in the case of *Ophioglossum,* this was not absolutely certain. As in the corresponding stage in *Ophioglossum,* above the egg cell, with its large and conspicuous nucleus, there is a clear space containing a small round body, which showed no evident nucleolus, but otherwise stained very much like the nucleus of the egg, and was probably the nucleus of the ventral canal cell (fig. 17, *D, v.*) While no certain cases of mitosis of the egg nucleus for the cutting off of the ventral canal cell were encountered, in one case the egg nucleus looked as if it were in the early prophase of division. Bruchmann's figures of the archegonium of *Botrychium lunaria* closely resemble that of *B. virginianum,* but he was unable to find a ventral canal cell, nor did he apparently see the division of the nucleus of the neck canal cell. The archegonium of *Helminthostachys* (fig. 11, *D*) closely resembles that of *Botrychium.*

<div align="center">FERTILIZATION.</div>

In only a few cases were spermatozoids seen within the neck and venter of the open archegonium in *Ophioglossum,* but twice a spermatozoid was seen within the nucleus of the egg; as there were no other stages obtained, however, the details of nuclear fusion could not be followed (fig. 16, *H*). The spermatozoid penetrates the nucleus of the egg, where for a time it can be seen distinctly. It is probable that its fusion with the egg nucleus is much like that described by Shaw for *Onoclea* (Shaw **1, 2**). In the mature egg cell the nuclear reticulum is often decidedly contracted, but whether this is normal or the result of reagents can not be said.

<div align="center">FERTILIZATION IN BOTRYCHIUM VIRGINIANUM.</div>

Jeffrey observed a single spermatozoid within the venter of the archegonium and noted that at the time of fertilization the egg developed what he called a "receptive prominence." I have observed the same phenomenon in several cases (plate 2, fig. 46), and in a good many instances have also found one or more spermatozoids within the venter. In one case, what looked very much like a spermatozoid was seen within the egg nucleus itself, and the process of fusion is probably very much the same as that so fully described by Shaw for *Onoclea.* In the specimen shown in fig. 17, *F,* one of the spermatozoids looks as if it has partially penetrated the egg, but it is impossible to be certain that it was not simply lying against it. In this case, just above the nulceus of the egg there was a slight break in the granular cytoplasm that looked as if it might be a receptive spot, but the spermatozoid was not entering the egg at this point.

In another instance a curious thing was noted, but whether it was normal or not it is impossible to say (plate 2, fig. 46). A spermatozoid had just entered the neck of the open archegonium and the egg had developed a very conspicuous receptive prominence. The nuclear contents were very strongly contracted and deeply stained and a portion apparently projected beyond the nuclear membrane

into the receptive prominence. A similar synapsis was noted in several cases. In most instances where free spermatozoids were present in the venter of the archegonium the egg nucleus presented a curious appearance (plate 2, figs. 47, 48). A single large nucleolus was present, but scattered through the nucleus there were sometimes a dozen or more of intensely staining round bodies, which at first sight looked like nucleoli, but on more careful examination were seen to differ from the large nucleolus in that they appeared less homogeneous and generally showed a central vacuole-like structure. These granules stain very much like the body of the spermatozoid, and it was thought that possibly they might be derived from a fragmentation of the body of the spermatozoid that had entered the nucleus, but this could not be satisfactorily demonstrated and the nature of this phenomenon must remain for the present uncertain.

Fertilization in *Helminthostachys* has not been observed.

## SIGNIFICANCE OF THE ENDOPHYTE.

That the presence of the endophyte is essential to the existence of the saprophytic gametophyte of the Ophioglossaceæ is indicated by the failure of the germinating spores to develop unless they become associated with the fungus. Moreover, the universal occurrence of a similar endophyte in all humus saprophytes among the seed plants indicates that in all of these chlorophyll-less plants the presence of the fungus is necessary for the existence of the host. Although it has not been directly proved, it is generally assumed that one rôle of the endophyte is the elaboration of some of the carbonaceous constituents of the humus. The infrequent communication between the external hyphæ and the internal mycelium makes it unlikely that the nutritive products are directly absorbed by the fungus, and it seems much more probable that the rhizoids of the gametophyte are the direct agents of absorption. How the humus constituents are changed by the action of the fungus so that they are available for the cells of the host is not clear, and it is by no means impossible that some at least of the necessary carbon may be derived from the fungus itself, in the digestive process to which it is subjected in the cells of the host. This seems plausible from the fact that in the green prothallia of certain ferns, where presumably the gametophyte is entirely able to supply its own carbon compounds through photosynthesis, these digestive cells appear to be wanting; at any rate they were not observed in a number of forms that I have studied.

The experiments of Ternetz (Charlotte Ternetz 1), show that certain fungi, including endophytic mycorrhizæ, are able to assimilate free nitrogen and confirm the assumption of earlier observers that the fungus is useful to the host in supplying it with nitrogen compounds; but, while this is probably a very important part of its functions, it seems to me that it is not perhaps the only one, and that the carbon also is supplied, directly or indirectly, through the agency of the fungus.

In an extended study of the endophytic mycorrhiza of the saprophytic orchid, *Neottia*, W. Magnus (W. Magnus 1) has shown that two types of mycelium exhibited by the endophyte are of very different nature. The slender, cylindrical hyphæ constitute the active portion of the fungus, which behaves like a parasite toward the cells which it invades, destroying the starch and probably other constituents of the cells, but not attacking the nucleus. The swollen vesicular mycelium, however, is a degenerating structure and is itself destroyed by the cells of the host, which actually digest these fungus mycelia in much the same way that the cells of the leaf of *Drosera* digest their prey. Magnus has very graphically shown that the relation of the two symbionts is mutually antagonistic, each one acting as a parasite on the other; nevertheless the presence of the fungus is essential to the higher

  
organism, so long as the latter is destitute of chlorophyll. The explanation of the widespread saprophytism exhibited by so many of the higher plants may be sought in this attempt to defend themselves against what was probably at first a strictly parasitic organism. Having acquired the power to attack and to feed upon the parasite, the photosynthetic functions were more and more subordinated, until a state of true parasitism (or saprophytism) resulted. The numerous semi-saprophytes like most of the green Ericales and many of the green Orchidaceæ, are good examples of transition stages, while the characteristic leafless humus saprophytes, such as *Monotropa* and *Corallorhiza*, represent the fully developed phase of this peculiar form of symbiosis. We might say that such green prothallia as those of the Marattiaceæ and *Gleichenia*, which contain an endophytic fungus, bear somewhat the same relation to the subterranean prothallia of the Ophioglossaceæ that the green Ericales do to *Monotropa*.

The occurrence of a similar endophyte has also been noted in a number of liverworts. Cavers (Cavers 1) has studied this association with some care in the common liverwort, *Fegatella*, as well as in some other Hepaticæ. He found in *Fegatella* that the endophyte is beneficial to the growth of the host, which was more vigorous when the fungus was present. He assumed that this was due to the assistance given by the fungus in the assimilation of organic matter from humus or other organic substrata. (See also Humphrey 1).

This occurrence of an endophyte in the Hepaticæ makes its occurrence in the green prothallia of ferns readily comprehensible. Whether in the latter it is an advantage to the host to have the endophyte present remains to be seen, but it is highly probable that such is the case. Once having acquired the habit of associating itself with the fungus, the gradual evolution of the purely saprophytic subterranean gametophyte of the Ophioglossaceæ from green forms similar to those of the Marattiaceæ is readily comprehensible.

## II. THE EMBRYO.

The development of the embryo in the Ophioglossaceæ has been more or less completely studied in *Ophioglossum pedunculosum, O. vulgatum, O. moluccanum, O. pendulum, Botrychium virginianum, B. lunaria*, and *B. obliquum* (Mettenius **1**, Bruchmann **1** and **2**, Lang **1**, Campbell **8**, Jeffrey **1**, Lyon **1**).

The first division in the young embryo in all of these is usually approximately transverse, although there may be a good deal of variation in this respect. It is probable that in all cases the primary root, the stem apex, and the foot all arise from the epibasal region. The embryo reaches a very large size before the root emerges from the overlying prothallial tissue and all of the organs of the young sporophyte are very late in developing, so that it is not easy to trace their origin back to the early cell divisions in the young embryo. Much the most conspicuous organ of the young sporophyte is the root, which may reach a very large size and an advanced stage of development before any evidence of the other organs is apparent. Indeed, several roots may be developed before the shoot is established. In *Ophioglossum vulgatum* and *Botrychium lunaria*, according to Bruchmann's account, the young
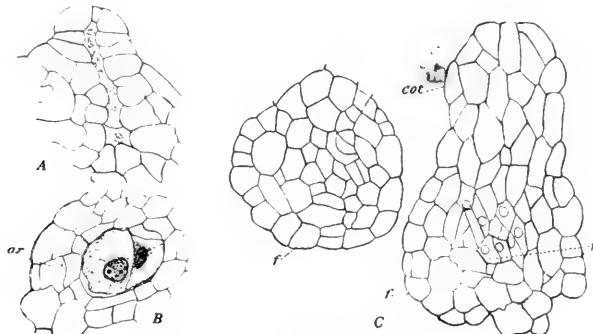


FIG. 18.—*Ophioglossum moluccanum.*

A. An old archegonium. ×180.
B. Two-celled embryo within the archegonium. ×180.
C. Two sections of an older embryo. ×180. *cot*, cotyledon; *f*, foot; *r*, root.

sporophyte remains several years under ground before the first green leaf appears above the earth, and it is probable that in *Ophioglossum pendulum* there is also a long period of underground existence before the first green leaf is developed. In *Ophioglossum moluccanum*, however, and in *Botrychium virginianum*, the first leaf developed is a green foliage leaf, which grows rapidly and soon appears above the surface of the ground.

### THE EMBRYO OF OPHIOGLOSSUM.

The first figures that we have of the embryo of *Ophioglossum* are those of Mettenius, but his figures of the embryo of *O. pedunculosum* are not at all satisfactory, although he shows correctly sections of the older sporophyte. Lang figures only one embryo, a somewhat advanced one of *O. pendulum*. Bruchmann figures a two-celled stage and a single more advanced embryo of *O. vulgatum*, but he describes and figures several stages of the young sporophyte. My own study of *Ophioglossum* was based mainly upon the development of the embryo in *O. pendulum*, where a

fairly complete series of embryos was obtained and the development followed quite satisfactorily.

In *O. moluccanum* I found two quite young embryos, the youngest consisting of two cells (fig. 18, *B*), the other much more advanced, with the cotyledon already differentiated and the beginning of the primary root recognizable. In the first embryo the basal wall was nearly transverse to the axis of the archegonium and, to judge from the arrangement of the organs of the older embryo, it looks as if the whole of the hypobasal region formed the foot, the epibasal half giving rise to the cotyledon and root. The older embryo (fig. 18, *C*) was cut longitudinally in the plane of the cotyledon, which at this time comprises pretty much the whole of the upper part of the embryo, the hypobasal region being occupied mainly by large cells which constitute the foot. Unfortunately, in this series the section containing the apex of the leaf was missing, and so it is impossible to say whether at this stage a definite apical cell is present in the cotyledon; but, as in somewhat older stages such a cell seemed to be always present, it is probable that there was an apical cell in the cotyledon of the embryo in question. At the base of the leaf, and almost in the center of the embryo, there was a group of actively growing cells, evidently marking the position of the root apex, which arises deep in the tissue of the embryo, very much as we shall see to be the case in the Marattiaceæ; and it seems probable that in *O. moluccanum*, as in *Danæa*, the root grows downward through the foot and in a direction coincident with the axis of the young cotyledon.
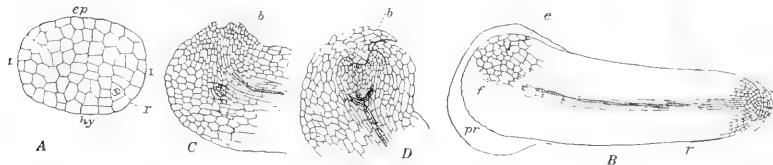


FIG. 19.—*Ophioglossum vulgatum* (after Bruchmann).

A. Median section of a young embryo. ×160. B. An older embryo. ×25.
C, D. Older embryos, showing beginning of apical bud, *b*; *r*, root. ×25.

The cotyledon grows upward and soon ruptures the overlying prothallial tissue, while the root grows down in the opposite direction and pierces the prothallium at a point some distance below where the leaf emerges. As the root grows downward through the foot the latter becomes unrecognizable, its outer cells remaining, however, as a zone of large cells encircling the equatorial region of the very much elongated bipolar embryo.

The young root very early develops a tetrahedral apical cell, like that of the later roots. This cell is probably cut out from the central tissue of the embryo, close to the base of the leaf. The cell *r* in fig. 18, *C*, to judge from its position and the arrangement of the cells around it, is probably the initial cell of the primary root.

The young cotyledon grows rapidly and has first a conical form (fig. 23, *B*), terminating in a definite apical cell. As it grows it develops a small oval lamina and a slender petiole, and presently the little green leaf appears above the surface of the earth.

The embryo of *O. vulgatum* differs remarkably from that of *O. moluccanum* in the late development of the leaf. Bruchmann was unable to obtain the younger stages of the embryo, so that the origin of the different members is still somewhat obscure. In this species it is the root which develops first, and it soon becomes

exceedingly conspicuous. Bruchmann considers that the root and foot are both of hypobasal origin, but he bases this on a comparison with the true ferns rather than upon actual study of embryos, as he was unable to obtain embryos sufficiently young to demonstrate this, and all trace of the original divisions disappears before any sign of the stem and leaf is evident. It may be well questioned whether, as in *Botrychium* and *O. pendulum*, the foot does not take up the whole hypobasal region. It is not impossible that the position of the basal wall may also vary in *O. vulgatum*. From a comparison with the embryo of *O. pendulum*, I am inclined to assign more of the embryo of the former to the foot region than is done by Bruchmann.

In *O. vulgatum* there is, finally, a differentiation of the stem apex from the epibasal region, as in *Botrychium* and the true ferns, while in *O. moluccanum* there is no trace of a stem apex in the very young sporophyte, this developing later as a bud upon the first root.

From a study of *Ophioglossum moluccanum* and also of *O. pendulum* it is evident that the history of the young sporophyte in these species differs strikingly from that of the other Pteridophytes. In both of these species the definitive sporophyte always arises secondarily, as a bud upon the root, in the same way that adventitious buds are commonly formed upon the roots of the adult sporophyte. Bruchmann notes in *O. vulgatum* the very precocious development of the primary
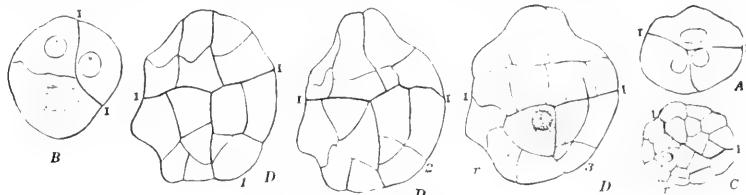


Fig. 20.—*Ophioglossum pendulum.*

A. Transverse section of very young embryo. ×180.
B. Longitudinal section of young embryo. ×180.
C. Three transverse sections of an older embryo; 1–1, basal wall; $r$, primary root. ×180.
D. An older embryo; $r$, primary root.

root and the late appearance of the stem apex and first leaf; but in this species the shoot apex, according to his statement, is derived directly from the epibasal half of the embryo, as it is in most Pteridophytes.

In *Ophioglossum pendulum*, where the development of the embryo seems to offer no check to the further growth of the prothallium, the position of the archegonium varies a good deal and it is impossible to tell from a section just what the position was in the living state, as the branches of the gametophyte extend in all directions and archegonia may be formed at any point upon their surface. To judge from the youngest stages of the embryo that were met with (fig. 20), the basal wall in this species is not necessarily transverse. In both of the cases figured it was oblique, and more nearly longitudinal than transverse. It is likely, however, that it is horizontal, or approximately so. In the four-celled embryo shown in fig. 20, *A*, the quadrant walls were at right angles to each other, and this was also the case in the five-celled embryo shown in fig. 20, *B*. Somewhat older embryos (*C*) show that there is a pretty regular octant formation, and Bruchmann states that this is also the case in *O. vulgatum*.

While in the typical ferns, and in *Botrychium*, all of the organs of the young sporophyte can be traced to certain regions of the young embryo, in *Ophioglossum*

*pendulum* only one of the definitive organs, the root, arises in this way, and this becomes differentiated at a very early period. One of the octants next to the archegonium at once becomes the apical cell of the young root. This cell is very soon recognizable by its size and shape, and quickly begins its regular segmentation. The primary cap-cell is soon cut off (fig. 20, *D*), and from now on the young root is very conspicuous. The octant divisions are very clearly marked in this case, and in section 3, which is the uppermost of the three, the large triangular apical cell of the root is very evident.

Two types of the embryo were seen in this species. One of these (fig. 21, *A*) is nearly globular in form; the other (fig. 21, *B, C*) is elongated. The former looks as if it originated from an embryo in which the basal wall was transverse to the axis of the archegonium; in the other it was probably more or less vertical. It is probable that in the former instance the root initial is one of the epibasal octants, while the whole of the hypobasal portion gives rise to the large foot. In the second type it is difficult to say which half should be considered epibasal and which hypobasal, but, as in the other case, one half may be considered to be root, the other half foot, the
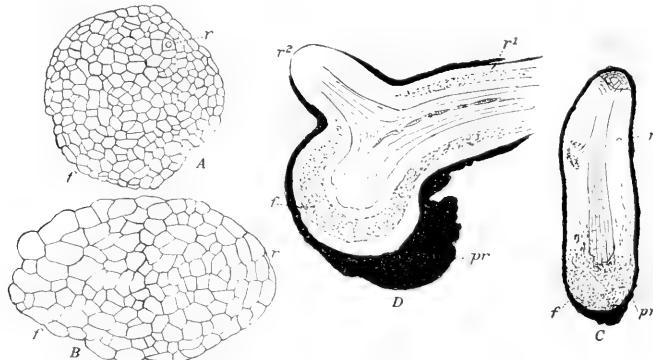


Fig. 21.—*Ophioglossum pendulum.* Older embryos.

A. Vertical longitudinal section; *f*, foot; *r*, primary root.
B. Horizontal section. ×75.
C. Older embryo, with single root.
D. Embryo with two roots; *pr*, gametophyte. Shaded region occupied by the mycorrhiza.

growth of both being nearly in a plane at right angles to that of the archegonium axis, and suggesting the relative positions of cotyledon and foot in the embryo of *Ophioglossum moluccanum.*

In form the first type somewhat recalls the embryo of *Botrychium virginianum.* The whole lower portion, or hypobasal half, forms the very conspicuous foot, while from the epibasal region the primary root is developing, and already the rudiment of the second root is visible. Whether the latter arises directly from the primary root, or whether it arises independently from the second epibasal quadrant, is not quite certain. The cells of the epibasal region are evidently actively growing, having abundant protoplasm and conspicuous nuclei. The cells of the foot are larger and much more transparent.

The second type of embryo (fig. 21, *B, C*) resembles very closely the second state of the embryo of *Ophioglossum vulgatum,* where, as is said by Bruchmann, the embryo is "all root."

In *O. moluccanum* the prothallium probably lives for a single season only, and the formation of the sporophyte stops its further development; but in *O. pendulum*, where embryos are much less frequently found, the large gametophyte continues its growth apparently unchecked by the development of the attached sporophyte, which retains its connection with the gametophyte for a very long time, as in *O. vulgatum* and *Botrychium virginianum*.

The embryo reaches a very large size before it breaks through the prothallium. The primary root then emerges as a conical point (fig. 3, *F*, *sp*). The second root remains short for a time. There seems to be a good deal of difference as to the time of the appearance of the latter. In the globular type of embryo the second root appears very early, and it looks as if it might have been formed quite independently of the primary root. Sometimes, however, the primary root may attain a length of several centimeters, and may even begin to form rootlets before the second root emerges; while in other cases the two roots grow in opposite directions and seem to be of about equal size (fig. 3, *E*).
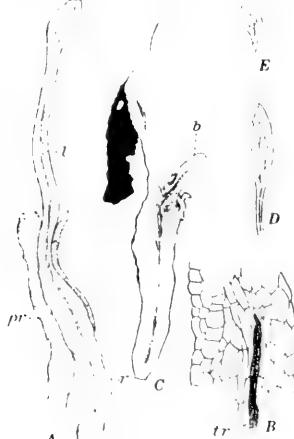


Fig. 22.—*Ophioglossum moluccanum.*

A. Median section of young sporophyte before formation of bud; *l*, cotyledon; *r*, root. *pr*, the gametophyte.  ×15.
B. Central region of same, more enlarged; *tr*, first tracheids.
C. An older sporophyte, showing bud, *b*, developing from primary root.  ×15.
D, E. Lamina of cotyledon, showing venation. ×3.

The leafy shoot in *Ophioglossum pendulum* does not appear until the root system is well advanced. The primary root, although attaining a length of 10 centimeters or more, in no cases showed any signs of leaf-bearing buds in the specimens that I collected. Rootlets were developed in some cases, and it is not impossible that there may be an extensive development of the root system before the first leafy bud is formed. This is quite in harmony with the large development of the roots in *O. vulgatum*, where Bruchmann believes that it may be eight or ten years before the first foliage leaf appears above the ground. In *O. vulgatum*, however, this leaf arises from the original stem apex derived directly from the embryo, and not from an adventitious bud.

*Ophioglossum moluccanum* and the similar species that grow with it differ very much from *Ophioglossum pendulum* in the character of the young sporophyte, which, as we have seen, at once develops a green foliage leaf or cotyledon. The sporophyte very closely resembles that of *O. pedunculosum* described by Mettenius; in fact, the resemblance is so close that it would seem to confirm the close relationship of this species and possibly its identity with some of the forms associated by Raciborski under the name *O. moluccanum* (Raciborski 1).

As Mettenius correctly showed in *O. pedunculosum*, the first organ to be developed is the cotyledon, which soon pierces the earth and appears as a green foliage leaf. This primary leaf is continued directly into the primary root, but no stem apex is developed, nor is any sheath formed about the leaf base in the young sporophyte, which consists practically of leaf and root alone. The latter often penetrates for some distance into the prothallial tissue before it emerges, so that the central portion of the young sporophyte is surrounded by a sheath formed by the prothallial tissue. A longitudinal section of the sporophyte (fig. 22, *A*) shows that the tissues of the leaf are continued directly into those of the primary root. A

single axial vascular bundle traverses the whole of the young sporophyte without interruption, and there is no recognizable boundary between the tissues of the leaf base and those of the root. The central region of the embryo is somewhat thicker and its outer cells are enlarged, these outer cells probably belonging to the foot through which the root has penetrated and which at this period can not be clearly recognized. The strictly bipolar character of the young sporophyte and the way in which it perforates the gametophyte resemble most nearly the corresponding stages of *Equisetum* and the Marattiaceæ, and it may be said that Lyon's recent studies on *Botrychium obliquum* indicate that in this species also there is a similar bipolar arrangement of leaf and root.

Mettenius pointed out in *O. pedunculosum* that the definitive sporophyte arises as an adventitious bud upon the primary root, either close to the leaf base or, more commonly, at some distance from it. This is also the case in *O. moluccanum* and the other allied species. In one case observed by me two independent sporophytes were found growing from the same prothallium, but this is unusual, and in most of the cases where two sporophytes seem to be present, one of these is really the secondary sporophyte growing from the primary root.
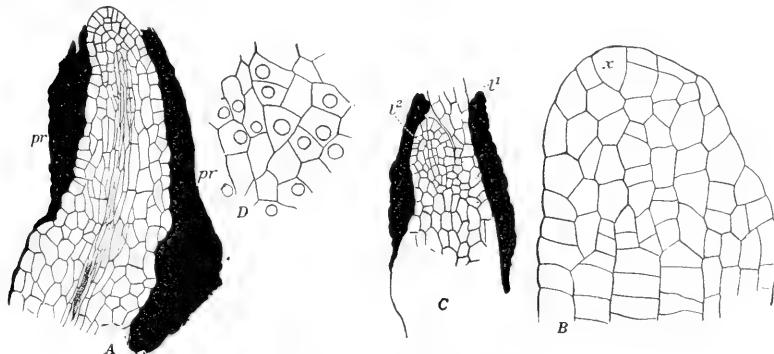


FIG. 23.—*Ophioglossum moluccanum.*

A. Young cotyledon, longitudinal section; *pr*, gametophyte. ×80.
B. Apex of cotyledon more highly magnified; *x*, apical cell.
C. Young sporophyte in which a rudimentary second leaf ($l^2$) is present. ×80.
D. Cross-section of apex of primary root. ×200.

While in *O. vulgatum* the sporophyte stays under ground for several years, in *O. moluccanum* there is every reason to believe that there is only a brief interval between the first formation of the leaf and its appearance above ground. The small size and the character of the gametophyte, as well as the quick germination of the spores and the rapid growth natural to a tropical climate, indicate that the gametophyte is an annual and that it dies as soon as the young sporophyte is established.

In *O. pendulum* the primary root as it breaks through the prothallium elongates rapidly, but just how far it grows before the bud is formed upon it could not be determined. These roots are very brittle and easily broken off, and in no cases were young buds found upon roots which were still connected with the prothallium. The first root sometimes reaches a length of 3 or 4 centimeters before the second root can be seen at all. The growth of the roots in the young sporophyte is in all respects like that occurring in the older ones. There is a large tetrahedral apical cell, the divisions of which are quite regular, and the conspicuous axial vascular cylinder is

developed at an early period. The stele of the second root joins that of the first where the latter joins the foot (fig. 21, *D*). The primary root in *O. pendulum* is, usually at least, diarch.

### DEVELOPMENT OF THE PRIMARY BUD IN OPHIOGLOSSUM MOLUCCANUM.

The several terrestrial species of *Ophioglossum* growing at Buitenzorg and associated under the name *O. moluccanum* differ strikingly from *O. vulgatum* in the further history of the young sporophyte as well as in the early development of the first functional leaf. This difference is also found in the young sporophyte of *O. reticulatum* ( ? ) collected at Hakgala in Ceylon. All of these tropical species agree with *O. pedunculosum*, which was correctly described by Mettenius (Mettenius 1), in the origin of the definitive axis which arises as an adventitious bud from the primary root of the young sporophyte. A similar condition of things probably is true also in *O. pendulum*, although in the latter no leaf is at first developed from the young sporophyte, which is composed exclusively of one or two roots in addition to the foot, so that the first leaf in *O. pendulum* is also an adventitious structure. Whether or not the primary leaf in *O. pendulum*, and probably at the same time the stem apex, arises as a bud from the primary root, or whether (which seems more likely) it is developed from a secondary root, can not be stated, as no young leafy buds were found in connection with the gametophyte.
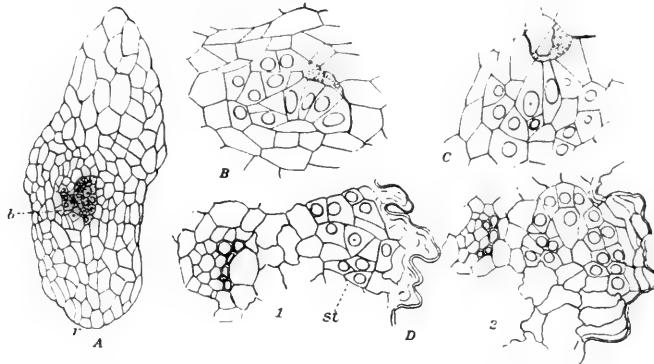


Fig. 24.—*Ophioglossum moluccanum.*
A. Tangential section of primary root, showing young, endogenous bud, *b*.  × about 50.
B. C. Two sections of the same bud.  B, the first leaf;  C, stem apex.  ×150.
D. Two transverse sections of a young bud;  1 passes through stem apex *st*;  2, through leaf.

Mettenius gives no detailed study of the development of the bud upon the root, nor does he state whether he recognized its endogenous origin. The bud arises much in the same way that a secondary root does, and is not visible upon the outside of the root until it is far advanced in its development, the young leaf breaking through the overlying tissues in much the same way that a young root emerges. In two instances the rudiment of the young bud could be seen close to the apical meristem of the root, in a position which is exactly the same as that described for the leafy buds formed at the apex of the root in *O. vulgatum*. More commonly, the bud originated nearer the base of the root, but it may arise at any point between the base and the apex. Figure 24 shows sections of the youngest buds that were found. *A* is a tangential section of the primary root, in which the young bud has formed at a point not very far back from the root apex, *r*. The first leaf of the young

bud and the young stem apex can already be recognized, and these have apparently developed quite independently of each other. The leaf rudiment, $B$, lies nearer the bundle of the main root and may perhaps have taken its origin from a single endodermal cell, but this could not be certainly determined. The young leaf soon forms a slightly projecting conical body, composed of a few cells with conspicuous nuclei, separated from the adjacent root tissue by a small space. At this stage it is not quite clear whether the apical cell has been developed, but later stages show the presence of an unmistakable apical cell of approximately tetrahedral form. Lying nearer the periphery of the root is the young stem apex, composed of a small group of meristematic cells, one of which is unmistakably the initial cell for the stem. This apical cell is truncate below and the base is broader than the outer free face. Above the stem apex can be seen the cavity which separates the outer tissues of the young bud from the cortical tissue of the root.

Fig. 24, $D$, shows two transverse sections of a bud of about the same age as the one just described. No. 1 passes through the stem apex and shows the conspicuous triangular apical cell; No. 2 shows a section of the leaf above the level of the stem apex with the basal tissue of the leaf extending around the stem region. This is the beginning of the hollow stipular sheath which incloses the stem apex of the bud and
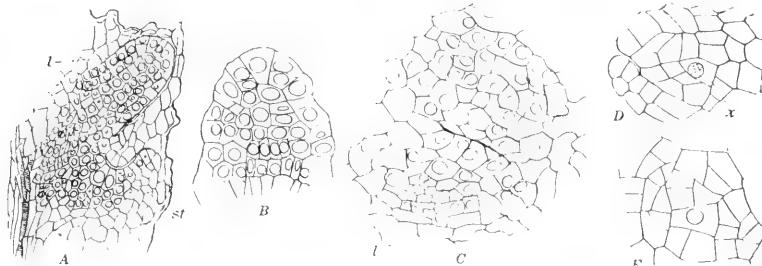


Fig. 25.—*Ophioglossum moluccanum.*

A. Bud about to break through primary root; *l*, first leaf; *st*, stem apex. × about 80.
B. Apex of leaf more highly magnified.
C. Transverse section of a young bud passing through base of leaf, *l*. ×150.
D. Section of same passing through stem apex, *x*.
E. Transverse section of stem apex from an older sporophyte. The apical cell is four-sided.

which is so conspicuous a feature in the stem apex of the older sporophyte. Probably the large central cell in the section of the leaf shown in No. 2 is the apical cell, but this is not quite certain.

A transverse section of a somewhat older bud (fig. 25, $C$) shows a narrow cleft at the base of the leaf in front, opening into a cavity within which the stem apex lies. It is impossible to say exactly how much of this stipular sheath really belongs to the leaf base and how much is derived directly from the adjacent cortical tissue of the root, since the tissues of the leaf base merge insensibly into the latter. From this time on, each new leaf develops this conical stipular sheath, which incloses the stem apex and the next youngest leaf, and which has so often been described in the older sporophyte.

If a median section of the young bud is examined just before the bud breaks through the overlying tissues of the root (fig. 25, $A$), it can be clearly seen that the leaf, which now has the form of an elongated cone, lies above the level of the young stem apex, and by this time the differentiation of the vascular bundle of the leaf is well advanced. This bundle connects with the vascular bundle of the primary root

and there is no connection between it and the tissues of the stem apex of the bud. The latter consists of a shallow mass of tissue with the conspicuous apical cell in the center, but below it there is no sign of the development of any procambium. The stem apex lies in a depression formed by a shallow ridge, which encircles it and forms the beginning of the stipular sheath belonging to the base of the leaf. The exact limits of the basal tissue of the leaf, as we have already stated, can not be clearly defined.    The cavity above the stem apex is still very evident, but how much of the tissue lying above the cavity belongs to the leaf base, and how much to the cortical tissue, is not clear.  The section through the apex of the leaf (fig. 25, *B*) shows a single large terminal cell which, without doubt, is the apical cell.

The young bud is now ready to emerge and, very soon after, the rapidly elongating apex of the young leaf pushes through the outer root tissue and emerges upon the outside.  The stem apex remains buried within the root tissues and the sheathing base of the leaf.  The leaf base is surrounded by a ragged sheath, formed by the ruptured outer tissues of the root.

The development of the bud upon the root in *O. pendulum* unfortunately could not be followed.  The smallest leaves found were 10 centimeters or more in length, and, although these were probably the primary leaves, they were not recognized as such at the time the plants were collected, and so there was no opportunity of tracing their connection with the original root.

The fully developed cotyledon in what may be considered the typical form of *O. moluccanum* is more or less lanceolate in outline (fig. 22, *D*).  There is a central vein from which branch secondary veins on either side, connecting with the central vein by anastomosing branches which inclose elongated meshes.  In the form with broader leaves, probably another species, the mid-vein is more obscure and the meshes are broader and more numerous.

Three types of the embryo may be recognized in *Ophioglossum*, represented respectively by *O. moluccanum*, *O. vulgatum*, and *O. pendulum*.  If, as seems not unlikely, *O. moluccanum* is the most primitive of the three, some interesting points arise as to the significance of the peculiarities exhibited by the embryo, which shows only two organs aside from the foot, viz, the cotyledon and primary root, these growing in an almost exactly opposite direction, without any clear line of demarcation between them.  I have ventured to draw a comparison between the sporophyte of *Ophioglossum* and that of *Anthoceros*, assuming that the former has arisen from some bryophytic type not unlike *Anthoceros*, by the development of a root from the base of the sporogonium and of a special foliar organ from the basal meristem of such a sporogonium.  The embryo of *O. moluccanum* approaches this hypothetical form, as it consists only of leaf and root, and no stem apex is developed from it, its growth being of limited duration.  In this case the definitive sporophyte is a secondary structure developed as a bud upon the primary root.  In *O. vulgatum*, however, the definitive stem apex, although of very late origin, is apparently a product of the original embryonic tissue, but the first foliage leaf is of much later origin.  In *O. pendulum* the formation of the leafy sporophyte is also secondary, but neither stem apex nor leaf is developed from the embryo itself.

If, as the writer believes, *Ophioglossum* represents the most primitive type of the fern series, it is quite conceivable that in *O. moluccanum* and its allies the embryo represents the condition existing in the ancestral type from which these have sprung. On the supposition that the leafy sporophyte is derived from a large bryophytic sporogonium resembling that of *Anthoceros*, there must have been a stage when the sporophyte consisted of two parts only, the upper sporogenous portion, which later developed into a sporophyll, as represented in *Ophioglossum*, and the root.  Of

course, it is quite possible that the peculiar origin of the definitive sporophyte in *O. moluccanum* and *O. pedunculosum* is secondary, but this is by no means necessarily the case. However, it seems highly probable that the extraordinary development of the roots in *O. vulgatum* and *O. pendulum* and the protracted subterranean life in these species constitute a secondary phenomenon associated with the pronounced saprophytic life of the gametophyte.

The apex of the shoot lies in a narrow depression between the base of the leaf and a narrow ridge which extends around it on the side opposite the leaf base. This ridge is the beginning of the conical sheath characteristic of the shoot apex of the older sporophyte. A transverse section of the young apex at about this time shows that the apical cell is triangular in outline.

The first root of the young bud does not emerge until the leaf is nearly complete. From this time on the further growth is due to the activity of the stem apex, from which new leaves and roots presumably are developed in the same way as in other species that have been studied. How long it is before fertile leaves are formed was not ascertained, but in the rapidly growing species of a tropical climate it is likely that this takes place before long. The occurrence of very small fertile individuals (fig. 55, *B*) points to this.

### THE EMBRYO OF OPHIOGLOSSUM VULGATUM.

The embryo of *O. vulgatum* (Bruchmann 1) differs from that of both *O. pendulum* and *O. moluccanum* in its development, but in the earlier stages it closely resembles the former species. As in *O. pendulum*, the root is the first organ to be developed and it reaches a large size before there is any indication of the formation of a stem apex or cotyledon. Bruchmann states that the root apex arises from the hypobasal half of the embryo, but his figure (fig. 19, *A*) of the young embryo, where the root apex is just visible, closely resembles a corresponding stage in *O. pendulum*, where the root is certainly of epibasal origin; and, to judge from the elongated form of the two-celled embryo which Bruchmann figures, one is inclined to believe that the basal wall is really at right angles to the long axis of the embryo, and not parallel with it, as he figures, so that the whole of the hypobasal part might be interpreted as the foot, while the root would be of epibasal origin, as it is in *O. pendulum*.

The next stage figured by Bruchmann (fig. 19, *B*) is very much like the type of *O. pendulum*, where only one root was developed at first, and in this case also we are inclined to consider the whole of the enlarged base of the embryo as the foot.

Before the cotyledon and stem apex can be recognized the first tracheary tissue of the root has already been developed, and about the same time the apex of the second root can be seen arising near the base of the primary root (fig. 19, *C*).

The cotleydon arises near the base of the root, upon the upper side of the embryo, and forms a small conical protuberance, which grows from a definite apical cell. At the base of the cotyledon, on the side turned away from the root apex, a shallow depression is formed, and in this there may be seen a single large superficial cell, which is apparently the apical cell of the very limited stem apex. Inclosing the cotyledon and the stem apex is an elevated ridge which grows up about them and finally forms a sheath, which rapidly grows so as to include the bud within a cavity completely closed except for a narrow canal which opens outward.

While the cotyledon and stem apex are thus formed from superficial tissue in *O. vulgatum*, their early inclosure in the sheath suggests that we have to do with a condition intermediate between the completely endogenous shoot apex in *O. moluccanum* and the entirely exogenous shoot in *Botrychium*.

The second root arises quite independently of the stem apex, being developed below the insertion of the primary root, and its vascular bundle joins that of the first root near its base.

A rudimentary vascular bundle is developed within the cotyledon, and also joins the bundle of the main root near its base, but no bundle at all is developed within the stem. The cotyledon reaches only a very small size, but the second leaf soon appears nearly opposite the cotyledon.

The second leaf also develops at an early period a vascular bundle, which connects with the vascular bundle of the primary root near its point of junction with the second root, and this exactly resembles the arrangement of the bundles in the bud formed upon the root in *O. moluccanum*. It is evident that in the young sporophyte of *O. vulgatum*, as in *O. moluccanum*, the vascular system is made up entirely of the bundles of the roots and leaves, the stem itself having no proper stele.

The second leaf finally emerges and appears above ground as the first sterile green leaf of the young sporophyte. Bruchmann states that the second leaf does not appear above ground until five years after it is first formed, and he believes that the sporophyte, at the time its first leaf appears above ground, is nine to ten years of age.

The third leaf may be fertile, but this is not always the case.

### THE ANATOMY OF THE YOUNG SPOROPHYTE OF OPHIOGLOSSUM.

In *Ophioglossum pendulum* the young primary root soon breaks through the prothallium and elongates rapidly, but owing to its brittleness it is easily broken off, and it is impossible to state here just how far it develops before the bud is formed upon it. The development of the second root varies a good deal. The first root may reach a length of 3 or 4 centimeters before the second root can be seen at all. The growth of these earlier roots is in all respects similar to that of the later ones. There is a large tetrahedral apical cell whose divisions are quite regular, and there is soon visible the axial vascular bundle which extends for some distance into the foot, where it ends blindly. The vascular bundle of the second root joins the first at the junction of the latter with the foot (fig. 21, *D*).

The first tracheary tissue appears at the point of junction, and is made up of short, somewhat irregular, pointed tracheids with reticulate thickenings. From this point the development of the tracheary tissue proceeds toward the apex of the roots. The bundle is diarch, as is plainly seen in cross-sections (fig. 27, *E*). The endodermis is very clearly defined and the characteristic radial markings are extraordinarily clear, especially in sections treated with a double stain of safranine and gentian violet. The tracheary tissue is also beautifully differentiated by this stain. The bundle is slightly elliptical in form and the protoxylem elements appear at the foci of the elliptical section. The first appearance of the tracheary tissue is some distance back of the apex and the development proceeds rather slowly. In the oldest part of the roots



Fig. 26.

A. Longitudinal section of an older bud of *Ophioglossum moluccanum*. ×50. *st*, stem apex; *l¹* first leaf of bud; *r¹*, first root of the bud.
B. Stem apex of same. ×90.

examined the two xylem masses were unequal in size, the larger showing about half a dozen tracheids in cross-section, the smaller two or three. Whether the two protoxylems are ultimately joined by intermediate tracheal tissue, so as to form a continuous plate, as in the older roots of the adult plant, can not now be stated, but in no cases examined was this true, and it is not unlikely that in the primary root the two xylem masses are permanently separated. The cells of the foot, as usual, are more or less papillate where they are in contact with the tissue of the gameto-phyte. They early become infected with the endophyte, which probably makes its entrance from the prothallial tissue, and not from the outside. This point, however, is not perfectly clear. The infected area follows the growth of the young root, but leaves the apical tissues free.

In *O. moluccanum* the leaf is the first part of the young sporophyte to develop. In the larger embryos the leaf forms a conical body, merging into a nearly globular basal portion, which is the foot, and within this, probably near the junction of the epibasal and hypobasal halves of the embryo, the apical cell of the root is developed. The leaf now shows a definite apical cell, triangular in section and exhibiting a regular segmentation. The inner cells of the segments form the axial strand of tissue, which is continued through the embryo into the root. The limits of the two
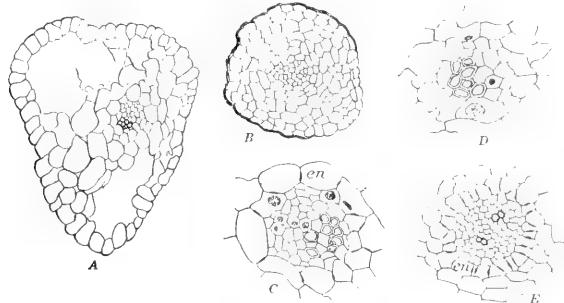


FIG. 27.

A. Section of petiole of cotyledon of *O. moluccanum*.
B. Section of young root.
C. Vascular bundle of median region of young sporophyte.
D. Vascular bundle of primary root.
E. Vascular bundle of primary root of *O. pendulum; en*, endodermis.

primary organs, the leaf and the root, remain quite indistinguishable. The central region, which remains surrounded by the prothallial tissue, is somewhat larger in diameter and the whole of this functions as a foot, although it is composed in part of tissue belonging to the root and the leaf. The young leaf elongates rapidly after it has ruptured the calyptra, and its apex begins to widen out, but still shows a single apical cell. As the upper part widens out there is a division of the original vascular cylinder, and there is developed within the leaf a reticulate system of vascular bundles or veins (fig. 22, *D*, *E*).

As already indicated, the vascular strand of the young sporophyte is continuous through the cotyledon and root, and sections at different points show essentially the same structure. The petiole of the cotyledon (fig. 27, *A*), which is traversed by two large lacunæ, shows that the axial bundle is decidedly collateral in structure. The xylem consists of a group of about half a dozen tracheids at the inner limit of the bundle, and no endodermis can be recognized. As a section of the bundle is made

in the mid-region of the sporophyte (fig. 27, C), the only difference noted is a slightly greater development of the xylem. The section of the root (fig. 27, D) presents almost exactly the same appearance as that of the leaf. Whether we should call the root bundle "monarch" or "collateral" is merely a question of terms. In the mid-region the endodermis can be clearly seen, and it is then evident that the xylem is separated from it by a single layer of pericycle cells.
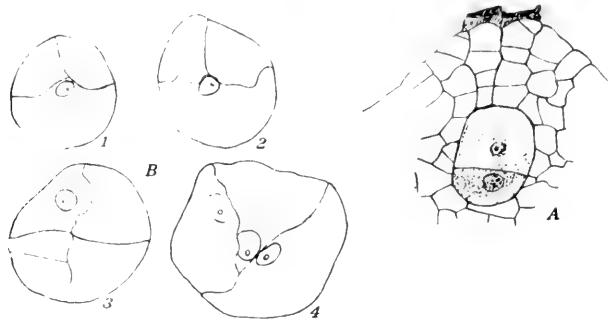


FIG. 28.

A. Archegonium of *Botrychium virginianum*, containing a two-celled embryo. ×275.
B. Four longitudinal sections of an embryo with 7 cells. ×275.

## THE EMBRYO OF BOTRYCHIUM.

The following account of the development of the embryo of *Botrychium* is based mainly upon material of *B. virginianum* furnished me through the kindness of Professor Jeffrey.

In this species, as in the other Eusporangiatæ, there is a marked increase in the size of the fertilized ovum before the first division takes place. At the time of the
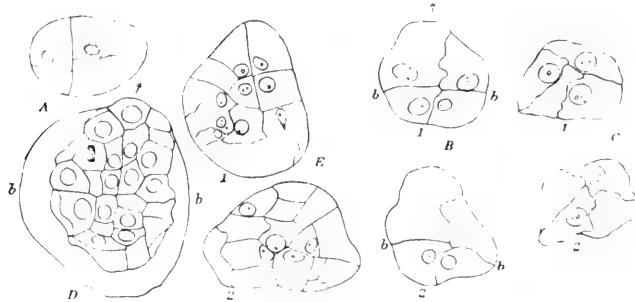


FIG. 29. –Young embryos of *Botrychium virginianum*.

Longitudinal sections. ×200. *b b*, basal wall. A. Four-celled embryo. B–D. Older stages.

first division the ovum is generally more or less elongated, but this is not always the case. This elongation is less marked in *B. lunaria* (fig. 36), while in *B. obliquum* the ovum becomes much elongated before the first division occurs (see Bower 9, fig. 266).

The first division wall, the basal wall, as in the other Eusporangiatæ, is, usually at least, transverse to the axis of the archegonium (fig. 28, *A*). Each of the cells thus formed is next divided by a vertical wall, so that the embryo is divided into approximately equal quadrants. Jeffrey states that the quadrants are next divided by a third (transverse) wall, so that there is a regular octant formation (Jeffrey 1, page 16), but adds that this segmentation presents various irregularities and this is confirmed by my own studies of the young embryo. In *B. lunaria*, according to Bruchmann, the octant divisions are very regular.

In *B. virginianum* the young embryo is often somewhat pointed above, and transverse divisions of such embryos may show that the octant formation is suppressed in the epibasal region, and this tapering upper part of the young embryo suggests a suspensor similar to that developed in *Danæa*, but less clearly defined, and probably not determined by the first division of the embryo. Whether the suspensor found by Lyon in *B. obliquum* (Lyon 1) originates in a similar fashion remains to be seen. This elongation of the upper part of the embryo, whether it



Fig. 30.—Three longitudinal sections of a young embryo of *Botrychium virginianum. r*, the root; *f*, the foot. ×200.

is considered as a suspensor or not, is doubtless, as Jeffrey has suggested, useful in pushing the developing embryo deeper down into the prothallial tissue.

For some time, only anticlinal walls are formed, this showing especially plainly in transverse section (fig. 31). Sooner or later periclinal walls are also formed, and it is possible that in some cases the first periclinal wall in one of the large epibasal cells may establish the initial cell of the root, but this is hard to decide. The early stages of the embryo are extremely difficult to embed without shrinkage, and this, together with the difficulty of securing a sufficient number of the right stages and the impossibility of regulating the direction in which they are cut, makes the determination of the origin of the primary organs of the young embryo a peculiarly difficult problem.



Fig. 31.—Four horizontal sections of a young embryo of *B. virginianum*.
1 is next the archegonium; 2 and 3 are nearly median sections.

Neither Jeffrey nor Bruchmann was able to recognize a root initial until the embryo had attained a large size, and its origin could not be determined. In the embryo shown in fig. 30, I am inclined to believe that the cell *r* is really the root initial. Unfortunately, stages between this and the one shown in fig. 32, where the root initial is unmistakable, were wanting, but the position and state of development of the root in the latter harmonize with this supposition. My fig. 32 is evidently of about the same size as Jeffrey's fig. 46, and probably an examination of other sections of the series from which Jeffrey's figure was drawn would show the root initial, as well as traces of the stem apex and cotyledon.

All of the organs of the young sporophyte arise, as Jeffrey showed, from the epibasal region, and in this respect *Botrychium virginianum* agrees with the Marattiaceæ and with *Ophioglossum*. According to Bruchmann, the embryo in *B. lunaria* remains quite undifferentiated up to the time it breaks through the calyptra, even the root apex being unrecognizable at this time. It is therefore impossible to say what relation the organs of the young sporophyte bear to the primary divisions of the embryo.
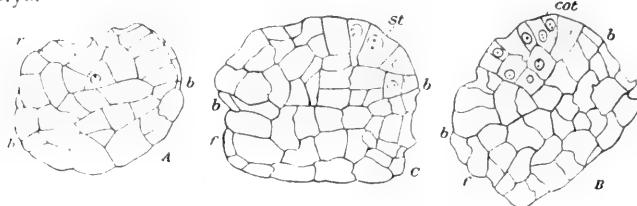


Fig. 32.—Three sections of an older embryo of *Botrychium virginianum*, cut transverse to long axis of embryo. *b b*, basal wall; *f*, foot; *r*, root; *st*, stem apex; *cot*, cotyledon.   ×200.

In *B. virginianum* traces of the quadrant formation are still evident at a comparatively late stage, and there seems no reason to doubt the correctness of Jeffrey's conclusion as to the epibasal origin of both the root and the cotyledon. As in *Ophioglossum pendulum* and *O. vulgatum*, the root is especially conspicuous and reaches a large size, while the stem apex and cotyledon are still inconspicuous. Indeed, in *B. lunaria*, according to Bruchmann, no trace of either stem apex or



Fig. 33.—Two sections of an older embryo of *Botrychium virginianum*, cut in the plane of the cotyledon.
*st*, stem apex; *r*, root; *f*, foot.   ×100.

cotyledon can be made out until the root has broken through the calyptra. At this stage (fig. 36, *C, D*) the embryo of *B. lunaria* bears a striking resemblance to that of *Ophioglossum vulgatum*.

It was found by Jeffrey in *B. virginianum* that the stem initial was developed before the cotyledon could be seen, but I have failed to verify this in the specimens I have examined. Fig. 32 shows three sections of a series taken from an embryo of about the age of Jeffrey's fig. 46. This embryo was cut transversely to its long axis, and the root apex is thus seen in cross-section. The root here probably comprises the whole of one of the epibasal quadrants, from the other of which, or from part of it, perhaps a single octant, the stem apex and cotyledon arise in close proximity. It is probable that part of this quadrant goes to form the "suspensor," or that epibasal tissue which is not concerned in the formation of the young organs of the embryo. Fig. 32, *B* shows a section passing through the young cotyledon, whose single apical cell is already differentiated. This in section is triangular, and the cell is probably of tetrahedral shape. Fig. 32, *C*, shows the section passing through the stem apex.

Probably the large cell (*st*) is the apical cell, but this is not absolutely certain. In form and position this cell resembles the apical cell figured by Jeffrey (Jeffrey 1, fig. 48) in an older embryo. It may be said that in Jeffrey's figure the beginning of the cotyledon, situated between the stem apex and the root, although not lettered, is evidently present.

As the embryo grows the root rapidly increases in length, and together with the foot comprises the greater part of the embryo. The foot is very large, usually nearly hemispherical in form, but not infrequently a good deal elongated (fig. 35), and penetrates deep into the prothallial tissue.

The apical cell of the root is very conspicuous and can be made out without difficulty as a large cell, triangular in form, whether it is viewed in longitudinal or transverse section. Segments are cut off in regular sequence from all of the four faces, and as these are relatively large and contain but little granular contents the apical meristem shows very clearly against the smaller celled and more deeply stained
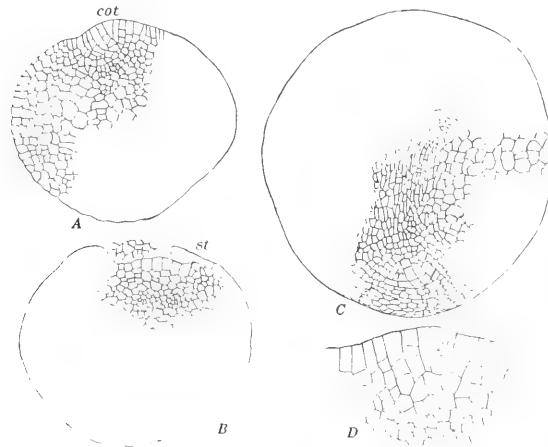


Fig. 34.

A-C. Three horizontal sections of an old embryo of *Botrychium virginianum*. ×75.
D. The stem region. ×150.

adjacent tissue. In the earlier stages the divisions in the segments cut off from the apical cell do not show absolute uniformity. The first wall in the young segment is probably anticlinal, and seems to be followed by a periclinal wall or walls, cutting off inner cells which contribute to the very large plerome cylinder of the young root. The root cap, which is very massive, is derived in part from segments cut off directly from the apical cell, and in part from cells separated by periclinal walls from the outer part of the lateral segments of the apical cell.

The development of the plerome begins very early, and it soon forms a conspicuous massive strand of procambium cells extending from the root apex to the junction of the root and foot, where it ends abruptly on the lower side, but bends upward on the upper side, and is extended as a simple axial strand of tissue into the cotyledon.

The cotyledon, which is first recognizable at an early stage in the development of the embryo, at the stage in question (fig. 35, *A*) projects slightly as a broad, flattened cone, strongly bent away from the root. Fig. 34, *A*, shows the cotyledon cut

4

through horizontally. At the slightly projecting apex there is a single apical cell, although it is not always quite certain which is the apical cell and which is its youngest segment. The broad base of the cotyledon is extended laterally, like the stipules of the older leaves, and there is thus inclosed a slightly depressed area, very much as in the embryo of *Ophioglossum vulgatum*, and within this is situated the stem apex, close to the base of the cotyledon, which bends over it so as to leave only a narrow space above the stem apex. A section across the base of the cotyledon shows a group of small cells indicating the section of the young vascular bundle which lower down (fig. 34, *C*) joins the young bundle of the primary root.

The relative position of the young organs just before the root ruptures the calyptra is best seen in a longitudinal section. Such a section (fig. 35, *A*) shows the very large foot, occupying approximately half of the embryo, and deeply embedded in the prothallium. Above this is the root, occupying the major part of the epibasal region of the embryo and varying somewhat in position, perhaps due to the early divisions in the young embryo. It may lie in a plane almost coincident with the cotyledon, or it may make a marked angle with the latter. In the former case the
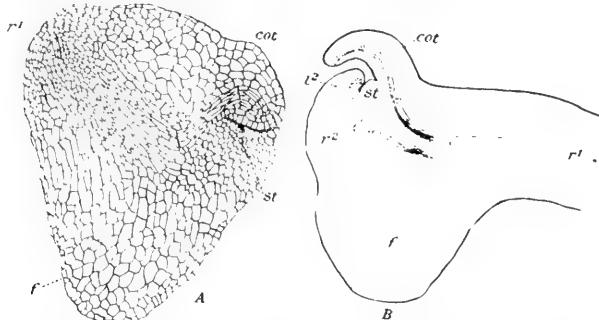


Fig. 35.

A. Median section of a young sporophyte of *Botrychium virginianum* about to emerge. × about 50.
B. An older stage; *l²*, second leaf; *r²*, second root. ×20.

young vascular cylinder is continued almost straight into the cotyledon; in the latter it bends sharply upward to meet it.

The stem apex, which is usually more or less oblique, is small and inconspicuous, but usually a single initial cell may be made out without difficulty, although sometimes it is not easy to distinguish this from the younger segments which have been cut off from it, as the apical meristem is very shallow, and in longitudinal section has the appearance of a columnar epithelium. There seems to be, at least in the earlier stages of development, some variation in the form of the initial cell, which may have a pointed base, or may be truncate below, and while later it usually shows the form of a three-sided pyramid, in these earlier stages it often approximates the truncate form found in *Ophioglossum moluccanum*.

The apical meristem of the stem is very limited in extent and, so far as could be made out, the segmentation at first does not follow any regular scheme. While in both root and cotyledon a central strand of procambium is developed at a very early period, *no trace of anything which can be interpreted as a "stele" is formed in the young stem*. The tissues derived from the further segmentation of the apical meristem remain undifferentiated parenchyma and contribute only to the central pith of the hollow woody cylinder which later traverses the axis of the young

sporophyte. In short, the strand of vascular tissue in the young sporophyte at the time the first root emerges is composed solely of the coalescent strands of the root and leaf exactly as is the case in the young sporophyte of *Ophioglossum moluccanum*, and there is absolutely no trace of a cauline stele.

The foot is composed of large parenchyma cells, which become smaller toward the periphery, where they usually show more or less dense contents and sometimes form a quite definite layer of epithelium-like cells, which stain much more deeply than the inner tissue of the foot. This outer layer of cells is presumably active in the absorption of nutriment from the adjacent prothallial tissue. Bruchmann states that previous to the emergence of the root of the young sporophyte of *B. lunaria* the cells of the embryo are densely packed with granular matter of apparently albuminous nature; but after the emergence of the root starch is developed abundantly in the cells of the foot. This appears to be true also of *B. virginianum*.

Compared with *B. virginianum*, the embryo of *B. lunaria* (fig. 36) is characterized by the lesser development of the foot and the later appearance and rudimentary character of the stem apex and the cotyledon.

The stem apex in *B. lunaria* (Bruchmann 2) is first evident as a slight superficial depression near the base of the massive root. A single superficial cell becomes differentiated as the apical cell of the shoot, and a small group of cells is formed before the rudimentary cotyledon develops. Both stem apex and cotyledon remain very inconspicuous. The cotyledon is developed as a scale-like rudiment, which never develops into a foliage leaf. Bruchmann states (Bruchmann 2, page 223) that from seven to nine of these rudimentary leaves are developed before the first green leaf appears above the earth. Hofmeister (Hofmeister 1) believed that three of these rudimentary leaves were developed during the first year, and that the second year the first spore-bearing leaf appeared above ground. Bruchmann thinks that this is not the case and that the first spore-bearing leaf requires five years for its development, as it does in the older sporophytes, and that only one leaf is formed each year, as in the older plant.

The first organ to penetrate the calyptra is, as we have seen, the root, which in *B. virginianum* is very large and quite overshadows the relatively inconspicuous bud at its base (fig. 7, *B*). At the time the root first emerges the vascular bundles still have the form of procambium, and it is not until the young root has a length of several millimeters (5 to 20 millimeters, according to Jeffrey) that the first tracheary tissue is developed. The first tracheary tissue arises at the base of the root and the development, as usual, proceeds toward the apex. The primary root in *Botrychium virginianum* is usually diarch, but triarch roots are sometimes found, and Jeffrey says that the size of the root has no relation to the number of protoxylems formed. The first tracheids are short and reticulately marked, as they are in *Ophioglossum*. The surface of the root is quite destitute of root hairs, as is the case in the adult sporophyte.

In *B. lunaria* the predominance of the root over the shoot is even more marked than in *B. virginianum*, for there are several roots, sometimes four to five, developed before the first foliage leaf is formed, and the bud remains extremely small and inconspicuous.

A longitudinal section of the cotyledon of *B. virginianum*, just before it breaks through the overlying calyptra, shows it to be strongly curved away from the root and overhanging the cleft within which lies the stem apex. If the section is exactly a median one, there may be seen near the tip, but lying somewhat toward the lower side, a marginal cell of triangular outline, which, from the arrangement of the cells about it, is evidently the apical cell. The cotyledon, instead of being straight as it

is in *Ophioglossum* and *Botrychium lunaria*, approaches the circinate form of that of the Marattiaceæ and the typical ferns. The young vascular bundle is now clearly evident, lying somewhat toward the inner side of the leaf, and it can be readily followed downward until it joins the root bundle, with which it is continuous.

In *B. virginianum*, while the cotyledon is still quite small, the second leaf appears close to the stem apex and nearly opposite the cotyledon, the sheathing base of which surrounds the stem apex, together with the young second leaf. The growth of the second leaf is also probably from a tetrahedral apical cell, and this is the case with all of the later leaves. While the second leaf is still very small, there begins the differentiation of the corresponding leaf trace, which joins the bundle of the primary root close to the point at which it gives off another bundle destined for the second root, the apical cell of which is cut out from the tissue near the base of the second leaf, at a point almost directly opposite the cotyledon. Indeed, the second root may be said to bear much the same relation to the second leaf that the primary root does to the cotyledon (fig. 35, *B*).
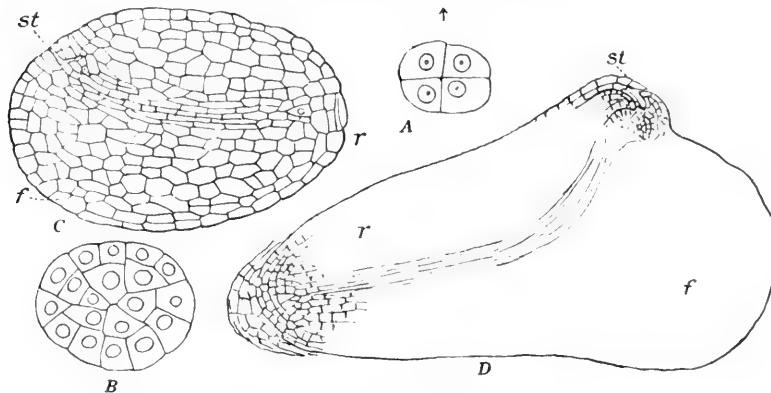


FIG. 36.—*Botrychium lunaria* (after Bruchmann).

A, B. Young embryos. ×225.
C, D. Older embryos. The cotyledon and stem apex, *st*, are developed much later than in *B. virginianum*.

The cotyledon in *B. virginianum* (fig. 7) is extraordinarily developed, reaching a size that probably is unequaled by any other fern. Its slender petiole reaches a length of 5 to 6 centimeters, and the lamina, unlike the simple oval primary leaf of *Ophioglossum*, is ternately divided and the segments deeply cut. The venation, instead of being reticulate as in *Ophioglossum*, is apparently pinnate, but this is really the result of an unequal dichotomy, as it is in the ultimate branches of the veins in most ferns.

The base of the petiole forms a stipular sheath like that found in the later leaves, and it is traversed by two vascular bundles, which unite into a single trace before joining the bundle of the root.

While in *B. lunaria* all of the early leaves remain rudimentary, and the first one to appear above ground is a fertile one, there are sometimes intermediate stages which may show a small ternate lamina and traces of the fertile spike, although they never appear above ground (see Bruchmann **2**, fig. 50).

If horizontal sections of the young sporophyte are examined, at about the time the cotyledon first emerges (fig. 41), the latter will be seen to have the form of a

crescent, the horns of which unite at about the level of the stem apex, so that the latter, together with the section of the second leaf, is completely inclosed by the sheath formed at the base of the cotyledon. The leaf trace in both the cotyledon and the second leaf is broadly oval in outline, increasing in breadth lower down, so that the section there becomes crescent-shaped and the two together form an imperfectly closed ring of small cells, the procambium of the future vascular cylinder of the axis, which clearly is formed entirely from the united leaf traces.

The apical cell of the stem appears triangular in cross-section, and from it are cut off regular segments, but, as we have seen from the study of the longitudinal section, the tissues which are developed from these segments below the apical cell remain undifferentiated and form merely a mass of large parenchyma cells with thin walls, which fill the space within the ring formed by the confluent leaf traces, and thus constitute the pith or medulla of the stem.

Jeffrey (Jeffrey 1, page 19) refers to the occurrence of short tracheids in the prothallial tissue in one case which came under his observation. There was some evidence that an embryo had been present, but it had disappeared, and he thought it possible that this was a case of apogamy, similar to that which has been repeatedly observed in *Pteris cretica* and in various other leptosporangiate ferns. If apogamy does really exist in *B. virginianum* it is the only case which is known among the Eusporangiatæ

*Botrychium obliquum* (Lyon 1) differs strikingly in the form of the embryo from either *B. virginianum* or *B. lunaria*, and these differences are so marked that Lyon proposes to separate *B. obliquum* and a number of allied species as a new genus, *Sceptridium*. The ovum in *Botrychium obliquum* becomes very much elongated after fertilization, and it is probable that the first wall separates the part next the archegonium from the inner cell, the former developing into a suspensor, very much as we shall see that it does in *Danæa*, and this suspensor becomes well developed and is conspicuous in the later stages of the embryo. Unfortunately, Lyon gives no details of the further history of the embryo until the definitive organs are far advanced. He states that the suspensor becomes elongated, pushing down the rest of the embryo, which he calls the "protocorm," believing that it is homologous with the so-called "protocorm" of *Lycopodium*—a resemblance which would probably disappear were a more thorough study of the subsequent history available. This development of a suspensor is the first case described in the ferns, but, as we shall see presently, a similar suspensor is regularly present in the genus *Danæa*, which in other respects shows some interesting resemblances to *B. obliquum* in the development of the embryo. Moreover, as we have seen, there is often an elongation of the upper cells of the young embryo of *B. virginianum*, which suggests a rudimentary suspensor.

Lyon figures only a very advanced stage of the embryo, at about the time when the root first emerges from the prothallium. At this time, aside from the presence of the suspensor, the most marked difference between *B. obliquum* and *B. virginianum* is the apparent absence of the foot in the former species, while this organ is so remarkably developed in *B. virginianum*. The root in *B. obliquum* penetrates the lower surface of the gametophyte, the cotyledon breaking through on the upper side, so that the relative position of the leaf and root is like that of *Ophioglossum moluccanum*, or the Marattiaceæ. In the orientation of the root and shoot, therefore, *B. obliquum* is much more like *Danæa* or *Ophioglossum moluccanum* than it is like *B. virginianum*.

It is to be hoped that further investigations will be made upon this extremely interesting species. We anticipate that when the history of the earlier stages is

known it will be found that a large foot is present in the embryo, as it is in the Marattiaceæ and the other Ophioglossaceæ, and the apparent absence of this foot in the older embryo is due to the root apex being formed deep in the central tissue of the embryo, as it is in the Marattiaceæ and in *Ophioglossum moluccanum*, and pushing its way downward through the foot, which thus becomes indistinguishable from the outer tissue of the root.

Except for the absence of the foot the organs of the older embryo of *B. obliquum* show the same relative positions as those of *B. virginianum*, and, as in the latter, the first leaf is apparently a functional green leaf which appears above the surface of the earth.

### THE EMBRYO OF HELMINTHOSTACHYS.

The embryogeny of *Helminthostachys*, the third genus of the Ophioglossaceæ, is unfortunately very incompletely known. Lang (Lang 1), to whom we owe the only published account of the gametophyte and young sporophyte, was unable to secure any young embryos and only very scanty material of the later stages. Lang says of the youngest forms which he found (Lang 1, page 40):

"The large hemispherical foot is deeply inserted in the tissue of the prothallium. The upper portion of the embryo had burst through the covering also of the latter; in it can be distinguished the primary root—the first leaf—and (covered over by the sheath of the first leaf) the depressed apex of the stem. The position of the organs is thus essentially similar to what is found in *Botrychium virginianum*."

Lang's material was collected in the Barrawa Reserve Forest, in Ceylon, and in February 1906 I visited the same locality and collected a large number of young sporophytes, many of which were still connected with the gametophyte; but none of the gametophytes were young enough to show young embryos. As we have already suggested, it seems very probable that the gametophyte of *Helminthostachys*, like that of *Ophioglossum moluccanum*, is annual and produces normally a single sporophyte, after which it perishes. The development of the gametophyte is probably dependent upon the annual inundation of the forest, and this perhaps accounts for the fact that all of the young sporophytes were of about the same age.

The youngest specimen which I found is shown in fig. 10. The petiole of the leaf was about 1 centimeter in length, and at the tip the minute lamina, strongly bent over like the young leaves of *Botrychium virginianum*, could be seen, showing the three lobes which characterize the fully expanded leaf. The cotyledon is rudimentary in *Helminthostachys*, the "cotyledon" described by Lang being really the second leaf. At the base, but separated from the root by a short internode, is a swelling which marks the position of the apical bud, inclosed within the hollow sheath at the base of the petiole. The root at this stage is still quite short, the first green leaf seeming to be more precocious than in *Botrychium* and thus resembling *Ophioglossum moluccanum*.

As the leaf grows the lamina expands and is seen to be ternate in form. Very often the two lateral lobes are of unequal size and show that the ternate form is due to an unequal dichotomy, such as is common in many ferns in the early leaves, which are transitional between the dichotomously divided cotyledon and the pinnate leaves of the older sporophyte. The ultimate divisions of the veins, as in *Botrychium*, are dichotomous.*

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* In a recent note (Lang 2) it is stated that a suspensor also occurs in *Helminthostachys*.

# III. THE YOUNG SPOROPHYTE.

## THE YOUNG SPOROPHYTE OF OPHIOGLOSSUM.

As we have seen, the single vascular strand in the young sporophyte of *Ophioglossum moluccanum* is continuous through the cotyledon and root, and sections at different heights show essentially the same structure throughout. The petiole of the cotyledon (figure 27, *A*), which is traversed by two conspicuous lacunæ, shows that the axial bundle has a perfect collateral structure. The xylem consists of a group of about a half dozen tracheids on the inner side of the bundle, of which the endodermis, however, is not evident. If a section made in the mid-region of the plant is examined, the only difference between its bundle and that within the petiole of the leaf consists in the somewhat greater development of the tracheary tissue. The section of the root bundle also almost exactly resembles that of the leaf. Whether we call the root bundle monarch or collateral is merely a question of terms.
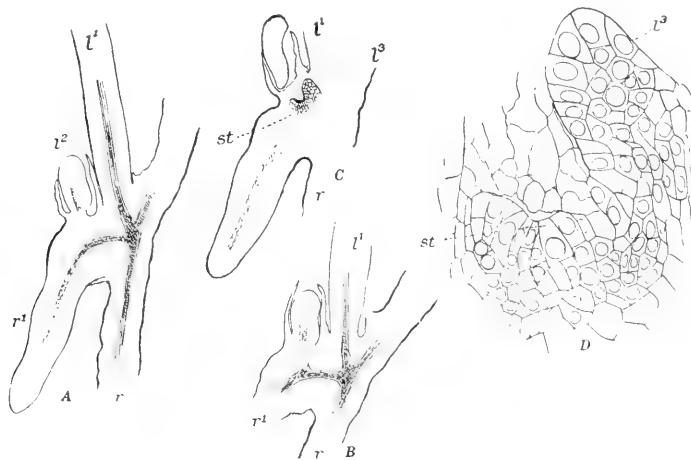


FIG. 37.

A–C. Three sections of a young sporophyte of *Ophioglossum moluccanum* with the first leaf, *l*¹, of bud fully developed; *r*, primary root of sporophyte; *r*¹, first root of bud; *l*², second leaf of bud.
D. The stem-apex more highly magnified, showing the third leaf, *l*³.

In the mid-region of the sporophyte the endodermis is well marked, and this is even more the case in the root. In the mid region the xylem is separated from the endodermis by a single layer of pericycle cells.

About the time that the leaf emerges the first root of the bud begins to develop, and, like the leaf, it seems to arise quite independently of the stem apex. The tetrahedral apical cell is cut out from the cortical tissue of the root some distance below the apex of the young bud, and as soon as the apical cell is established active segmentation begins in the tissue below the bud and a strand of procambium is developed, connecting the young root apex directly with the vascular bundle of the primary root. Thus the vascular bundles of both the first leaf and the first root of the young bud are directly connected with the primary root of the young sporophyte and have nothing to do with the tissues in the apex of the stem of the bud, which is still very small.

The two bundles, belonging respectively to the leaf and the root of the bud, join the bundle of the primary root very near together, and from near this point of junction the development of the tracheary tissue proceeds toward the apices of the young organs. The first tracheids, like those found in the median region of the young sporophyte, are short reticulate ones.

The first leaf of the bud grows rapidly and closely resembles the cotyledon in form and size, and at first it often looks as if two young sporophytes of equal size were growing from the prothallium, but a very slight examination shows that the second leaf belongs to the bud upon the primary root. The second leaf of the bud also seems to be formed independently of the stem apex and arises nearly opposite the first one (fig. 37, $A$). Its development is practically the same as that of the first leaf, and it pushes through the outer tissue of the root at a point removed by an appreciable distance from the base of the first leaf, from which it is separated by a sheath about the base of the latter, formed by the outer root tissues.

The vascular bundle in the second leaf is soon developed and can be followed down to the bundle of the first root of the bud, to which it bears somewhat the same relation that the bundle of the first leaf of the bud does to the primary root of the sporophyte. Fig. 37 will show clearly the arrangement of the vascular bundles in a young bud in which the first leaf is fully developed, while the second and third are pretty well advanced. The latter ($l'$) shows very plainly the conspicuous apical cell and the beginning of the leaf trace. The apical cell of the stem at this time is sufficiently conspicuous, but the amount of tissue surrounding it is quite limited in extent. In fig. 37, $D$, it is probable that the fourth leaf has begun to develop. Above the apex of the stem the cavity of the leaf sheath can be clearly seen. By this time the first root of the bud has emerged below the first leaf and the tracheary tissue is well developed in its basal region.

The second root of the bud originates below the apex of the stem in the vicinity of the base of the second leaf. The vascular strand of the third leaf joins it, and the third leaf seems to have somewhat the same relation to the second root that the second leaf does to the primary root.

The study of the development of the young bud shows that, up to the time of the production of the third leaf, the young organs arise quite independently of each other from the tissue of the primary root. The young sporophyte is, so to speak, made up by the union of several independent members. The third and fourth leaves arise from the stem apex as all of the later ones do, but the first and second leaves and the first root show no recognizable relation to the stem apex. Their vascular bundles are directly connected with the bundle of the primary root of the young sporophyte, and are in no way associated with the tissues which belong to the stem region of the bud. No material was available for the study of the origin of the leafy bud in *O. pendulum*, but the first leaf evidently develops very much later than it does in *O. moluccanum*. Young leaves 10 centimeters or more in length were found, which, there is some reason to suppose, were the primary leaves of the young plant; but they were not recognized as such at the time they were collected and it is therefore impossible to say whether they really were the first leaves developed. After the study had been made of the young sporophyte in *O. moluccanum*, and the secondary origin of the stem apex was made out, it was recognized that these small leaves in *O. pendulum* also were probably the primary leaves of the plant, but it was too late then to trace the connection of the roots from which they developed to their connection with the primary root of the young sporophyte.

I was unable to determine just how soon the fertile leaves are developed from the young sporophyte in *O. moluccanum*. In all of the specimens that were sectioned,

the first three or four leaves were sterile and practically like the cotyledon, and it is not certain which leaf, under ordinary circumstances, first gives rise to the spore-bearing spike. In *O. moluccanum* and the other tropical species of *Ophioglossum* growth is continuous, and it is evident that the development of the leaves does not take the long period required in *O. vulgatum* and other species of temperate regions, where growth is interrupted each year and where only one leaf is developed in the
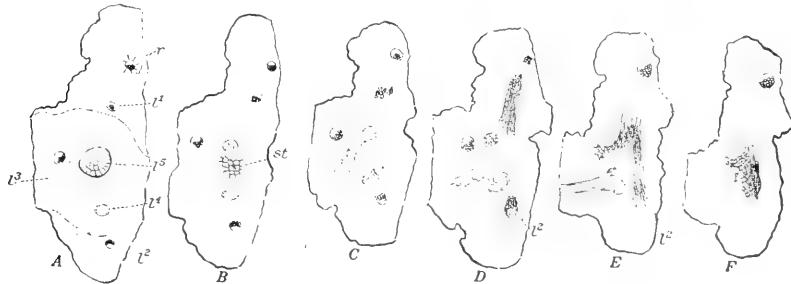


FIG. 38.

Six of a series of transverse sections of a young sporophyte of *Ophioglossum moluccanum*, still attached to primary root, *r*. Section B passes through the stem apex. ×35.

season. Mettenius states that *O. pedunculosum*, which, as we have seen, may perhaps be identical with *O. moluccanum*, develops three leaves each season. This was in the Botanical Garden at Leipzig, and it is highly probable, under the much more favorable conditions of its native tropical habitat, that this number would be exceeded. There was not time to make investigations in regard to this



FIG. 39.

A. Section of the sporophyte shown in fig. 38, showing arrangement of leaves; *r* is the primary root. × about 60.
B. Vascular bundle of first leaf, more highly magnified.
C. Stem apex, showing youngest leaf trace, $l^5$; *st*, apical cell of stem.

point, but it is very certain that several leaves are developed in the course of each year and that the development of the individual leaf does not require the long period necessary in the species of cold climates.

Figures 38 and 39 show several transverse sections forming a series taken from a young sporophyte with three fully developed leaves and two younger ones. This bud was developed from a small root, but it was not certain that this was the primary root of the embryo. The spirally arranged leaves show the two-fifths divergence. The second and third leaves of this bud were successively larger than the first leaf, but the section of the petiole showed a single centrally placed vascular bundle of the same type as that of the primary

leaf and all of these leaves were sterile, nor could any indication of the develop-
ment of a central spike be seen in the development of the fourth leaf. The first
leaf of the bud, like the cotyledon, showed two large lacunæ in the petiole, but in
the second leaf there was but a single one and this was interrupted at intervals,
while the petiole of the third leaf appeared almost solid, there being only small,
irregular, intercellular spaces, such as are always found in any loose parenchyma.
All of the vascular bundles are collateral. In the lower portion of the third leaf
eleven tracheids could be seen in the transverse section of the bundle, but higher
up the amount of tracheary tissue was reduced.

Figs. 38, $A$, and 39, $A$, were cut just above the stem apex and showed clearly
the arrangement of the first four leaves. The youngest leaf is still entirely surrounded
by the conical stipular sheath belonging to the third leaf. Fig. 38, $A$, is taken imme-
diately above the stem apex and passes through the very young fifth leaf. This
section shows very satisfactorily the arrangement of the first five leaves. Section $C$
lies a short distance below the stem apex. $r$ is the root from which the bud has
arisen, and the group of tracheids at its junction with the bud marks the point
of union of the bundles of the first leaf and the first root of the bud. In the center
of the section may be seen a mass of large-celled parenchyma, the central pith of the
stem, which is derived from the large-celled meristem of the stem apex. The broken
ring of procambium surrounding the pith is composed of obliquely cut traces of the
fourth and fifth leaves and the basal tissue of the second root of the bud. The leaf
traces from the second and third leaves are still free in the cortical tissue of the young
stem. $E$ and $F$ pass through the base of the bud. The stout bundle of the first
root is seen connected with the bundle of the root upon which the bud was developed,
and the second leaf trace ($l^2$) bends in to meet the bundle of the root but is cut
at a level above the point of junction. Close to the second leaf trace is the lower
part of the fourth leaf trace, which is connected with the trace of the second root
of the bud. The steles of both the first and second roots of the bud run horizon-
tally across its base before they emerge, and the leaf traces are almost perpendic-
ular to these. The second leaf trace at the base of the bud joins the stele of the
first root and the short tracheids curve out from the opposite side of the bundle of
the first root to unite with the base of the third leaf trace, which does not, as might
have been expected, show any very obvious relation to the second root. At the
point where the first root bends downward to emerge from the overlying cortical
tissue the tracheary tissue of this, with the bases of the leaf traces, forms a large
mass of irregular, broad, reticulate tracheids, occupying the center of the base of
the young bud. It was probably the presence of this mass of tracheary tissue at the
base of the bud which led Rostowzew to state that the bundle of the bud is at first
solid. It is possible that the buds formed on the old roots of $O.$ $vulgatum$ may be
somewhat different in structure from the early buds in $O.$ $moluccanum$ and that
there really may be such a solid stele at its base.

According to Rostowzew (Rostowzew 1, 2) and Poirault (Poirault 2), the buds
upon the older roots of $O.$ $vulgatum$ usually arise close to the apex, much as they
sometimes do in the formation of the bud upon the primary root of $O.$ $moluccanum$.
Van Tieghem thought that the apex of the root itself became changed into the
leafy shoot, but both Poirault and Rostowzew demonstrated that the young bud
originated from a segment of the apical cell of the root and not from the apical
cell itself; the latter continues its growth and the root thus grows beyond the
point of insertion of the young bud. Rostowzew states that an outer cell of the
segment divides into two superimposed layers, of which the superior one, or the
one nearest the root apex, gives rise to the stem apex of the bud, while from the lower

one, i. e., the one turned toward the base of the root, the first leaf arises. It thus appears that in these adventitious buds in *Ophioglossum vulgatum* there is much the same arrangement of the first organs of the young bud that we have seen to be the case in *O. moluccanum*, the leaf and the stem apex being virtually independent organs. From Rostowzew's figures it is evident that the bundle from the first leaf of the bud in *O. vulgatum* is connected directly with the main root in very much the same way as it is in the primary bud in *O. moluccanum;* but apparently the first root of the young bud is inserted higher up and does not connect directly with the primary root; this point, however, needs further elucidation. Moreover, according to his statements, there is a good deal of difference shown as to the time of the emergence of the leaves and roots, three or four roots sometimes being well developed before the first leaf expands. This reminds one of the behavior of the primary sporophyte, where Bruchmann found that several roots were developed before the first green leaf made its appearance.
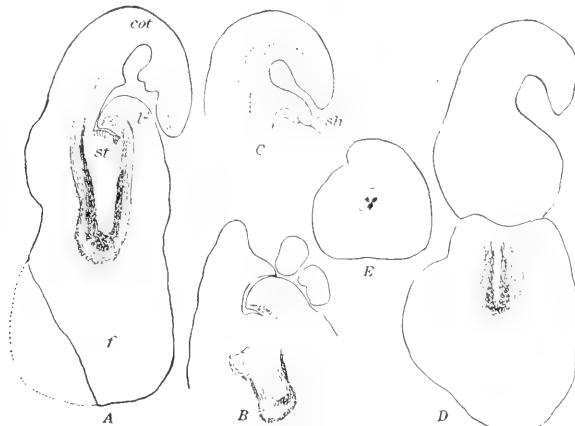


FIG. 40.—Vertical sections of a young sporophyte of *Botrychium virginianum*. The sections were cut at right angles to the axis of the root. *sh*, stipular sheath of cotyledon; E, section of primary root, which in this case was triarch. ×20.

## THE YOUNG SPOROPHYTE OF BOTRYCHIUM.

Jeffrey (Jeffrey 1) has described at some length the structure of the young sporophyte of *B. virginianum*, and Bruchmann (Bruchmann 2) has studied the earlier stages of the sporophyte in *B. lunaria*. The present account is based mainly upon my own studies of *B. virginianum*, made from material furnished me through the kindness of Professor Jeffrey. Jeffrey found that from the beginning the conspicuous stele found in the axis of the young sporophyte was a hollow cylinder, which he assumes to be a really cauline structure, and not made up of leaf traces. The results of my own studies indicate that, as in the case of *Ophioglossum*, the vascular system of the stem is made up exclusively of confluent leaf traces.

Fig. 35, *B*, shows a longitudinal section of a young sporophyte in which the first two leaves are evident and the continuation into these of the tissues of the vascular bundle of the root is very clear. The large central pith continues up into the stem apex, but the whole of the fibrovascular tissue is continued into the leaves, there

being no trace of procambium inside of the leaf traces in the apical region of the
stem itself. Fig. 40 shows a sporophyte which was cut at right angles to the primary
root, which in this case had a triarch bundle. Near its base the three xylem masses
increase in size and form a nearly complete ring of tracheary tissue, which higher
up is continued into the leaf traces. The third leaf has already begun to form, but
is still very small. Below it, however, can be clearly seen the beginning of its large
leaf trace, which can be followed downward to its junction with the second root,
which is now just about ready to emerge. The stele of the second root is con-
nected with the central cylinder of the axis near its junction with this third leaf
trace. The stem apex occupies a very small area, crowded between the base of the
second and third leaves. Its deep, narrow, apical cell is not as conspicuous as is
often the case.
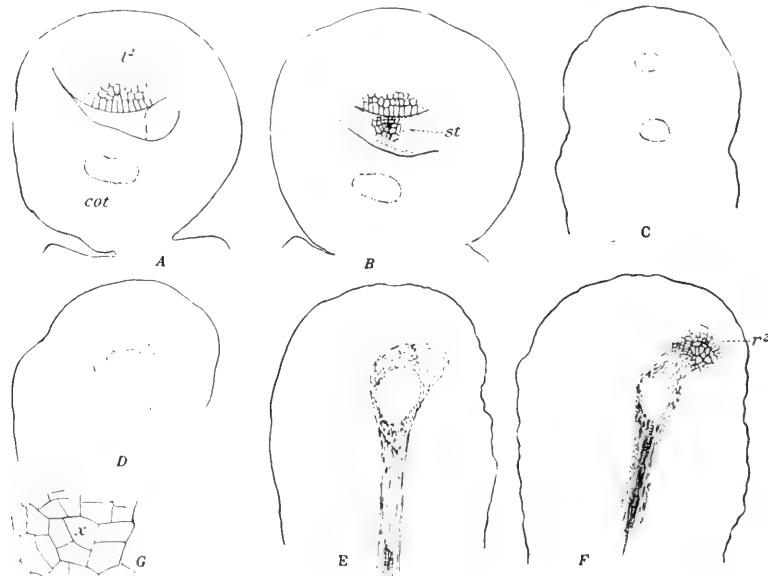


Fig. 41.

A-F. Six of a series of horizontal sections of a young sporophyte of *Botrychium virginianum*. X75.
B passes through stem apex, *st*, which is shown more enlarged in G; *r²*, second root; *l²*, second leaf.

A similar sporophyte is shown in cross-section in fig. 41. This was cut in
the plane of the first root, which makes a sharp angle with the base of the cotyledon.
The thick stele of the root is much expanded where it joins the leaf traces to form
the beginning of the tubular stele of the axis, and at the point of junction there is
a large nearly solid mass of short, irregularly disposed, reticulate tracheids. This
young sporophyte was evidently the further development of an embryo of the
type shown in fig. 35, *A*. The trace of the cotyledon makes a right angle with the
stele of the root and on the side opposite to its junction with the stele of the root
there can be seen the trace belonging to the second leaf, which is already well
developed. The apical cell is tetrahedral in *Botrychium virginianum* and the
cells are cut off in regular segments from its three lateral faces. Segmentation is

evidently slow and there is usually a good deal of difference in the stage of develop-
ment in the three segments composing a single cycle. The first division in each
of the lateral segments cuts off a small inner cell from a large outer one, and the
latter is then divided into two by an anticlinal wall. The divisions were not followed
beyond this stage and the limits of the segments comprising the second cycle can
not be easily recognized, as by this time the youngest leaf begins to grow and the
stem apex is thus crowded into a very small area between the bases of the two
youngest leaves. The shallow mass of meristematic tissue comprising the stem apex
merges gradually into the large-celled parenchyma which makes up the pith cylinder
inside the hollow stele of the axis.

Longitudinal sections of a much older sporophyte are shown in fig. 42.

Except for the vascular bundles, the whole of the tissue of the young sporophyte
is composed of large-celled parenchyma which, especially in the region belonging
to the foot, contains large quantities of starch.

The form of the young leaves in *B. virginianum* is very different from that
in *B. lunaria*. In the former the young leaf is bent forward over the stem apex and
the apical cell is at the tip of this bent-over portion. In *B. lunaria*, however, the
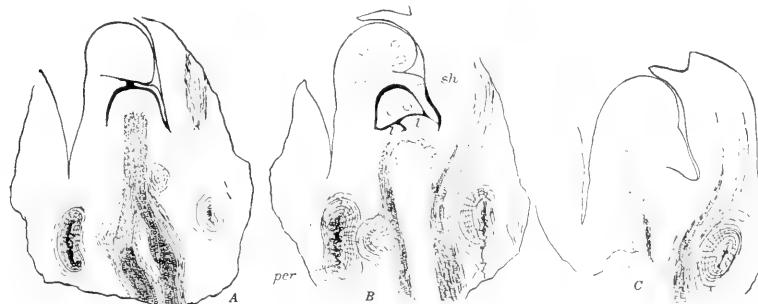


FIG. 42.

Three longitudinal sections of a young sporophyte of *Botrychium virginianum,* with several leaves.
*per,* absciss layer of periderm; *l, l,* the youngest leaves; *sh,* stipular sheath.   ×20.

bent-over portion becomes the stipular region and the apical cell of the young leaf
arises in the convex upper portion, so that the leaf grows straight upward instead
of being bent over as it is in *B. virginianum*. The latter species in this respect
resembles the Marattiaceæ.

The stipules in *B. virginianum* are lateral structures which extend around the
next youngest leaf and the stem apex, but the sheath has a narrow cleft in front, so
that we may really speak of two stipules instead of a single stipular sheath (fig.
42, *A*). In this respect also there is a strong resemblance to the Marattiaceæ.

Fig. 43 shows a series of transverse sections of a young sporophyte of *B. vir-
ginianum* in which the fourth leaf was just evident. Fig. 43, *A* and *B*, are near
the base of the fourth leaf and show the arrangement of the first four leaves. The
base of the cotyledon (*cot*) is a good deal flattened and the petiole is seen to be
traversed by very conspicuous lacunæ, reminding one of those in the cotyledon of
*Ophioglossum moluccanum*. The conspicuous stipules can be seen extending
partly around the group of younger leaves, but not completely inclosing them. The
two vascular bundles of the petiole are beginning to unite to form the single leaf
trace which is found lower down. The xylem in each bundle forms a broad band

of wood, which is surrounded by the phloem, although the latter is much less
developed upon the inner side than upon the outer and the bundles thus approach
the collateral condition found in the trace lower down. Small protophloem ele-
ments occur in the outer part of the bundle and are continued part way around
toward the lower side, to the point where the two bundles are beginning to fuse,
but here they are no longer visible.

The second leaf shows no lacunæ and the base of the petiole is more nearly
cylindrical than is that of the cotyledon. Like the latter, the petiole contains two
vascular bundles which at the level of this section were still quite distinct, and the
permanent elements were just beginning to form. The stipules in the second leaf
completely inclose the third, but there is a very narrow slit in front where the two
stipules meet. The next section figured is taken just above the stem apex and cuts
through the youngest (fourth) leaf. The two bundles of the petiole of the third leaf
are evidently still quite separate, but closely approximated, and as yet show no per-
manent tissue. The stipules of the third leaf are just becoming evident. At the
level of the stem apex the bases of the third and fourth leaves are completely united,
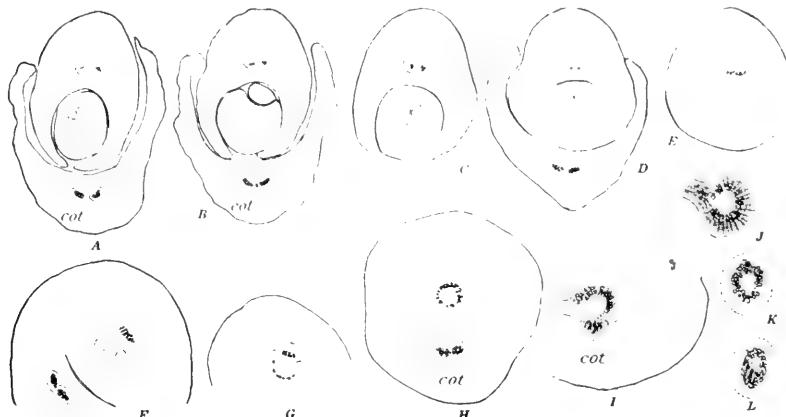


Fig. 43.

Series of transverse sections of a young sporophyte of *Botrychium virginianum*, showing union of leaf traces to form vascular
cylinder. D and E show the separated leaf traces which unite further down; K shows the bundle of the axis near
its junction with the stele of the primary root.

and the base of the second leaf is also partially fused with these. The two bundles
from the second leaf are now beginning to unite to form the single leaf trace, while
in the third leaf the process is complete and a single oval mass of procambium cells
marks the position of the single trace of this leaf. On the opposite side of the
apical meristem is a similar but less developed mass of procambium, representing
the trace from the young fourth leaf.

For some distance below the stem apex the two bundles of the third and fourth
leaf respectively are quite distinct, and are separated from each other by a mass of
large-celled pith. Lower down the two bundles approach and coalesce, and on the
side of the second leaf trace (which is turned away from the third one) a group of
actively dividing cells can be seen which forms the beginning of the fifth leaf trace,
although the leaf itself can not be recognized at the summit. These three masses
of procambial tissue, viz, the young traces from the third, fourth, and fifth leaves,

make a nearly continuous crescent-shaped mass of procambium. Fig. 44, *A*, *B*, shows the section of the central stele; and the space between the horns of the crescent, i. e., the space between the third and fifth leaf traces, constitutes the so-called "foliar gap" which faces the second leaf trace. The central cells of the young stele have already begun to form cambium, which in the older stem constitutes such a characteristic feature of the stele.

Before any tracheary tissue is formed in the young stele, a few thick-walled cells can be seen in the outer part of the portion of the stele corresponding to the third leaf trace. These are protophloem cells, and similar ones are developed later in the younger parts of the stele. The first tracheary tissue appears in the region of the steel section belonging to the third leaf trace. In the section shown (fig. 44, *C*)
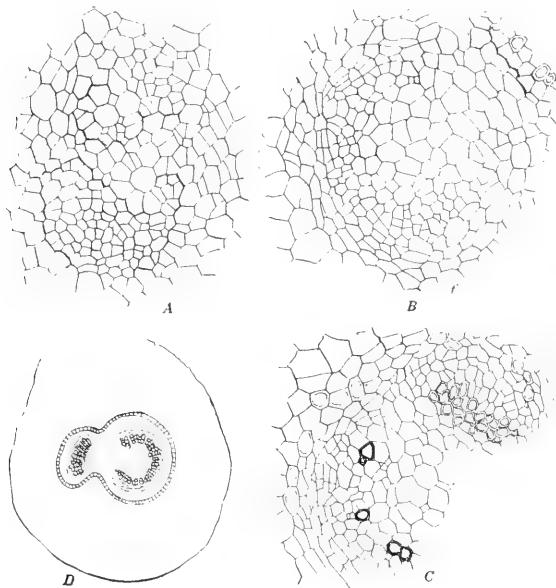


FIG. 44.

A, B, C. The young vascular bundles of sections E, F, G, of the series shown in fig. 43. ×150.
D. Section of a young sporophyte of *Botrychium virginianum*, showing junction of a leaf trace with stele of axis. ×75.

there are three groups of these primary tracheids, separated by a considerable interval and placed at the extreme inner limit of the young stele. In the older portions of the bundle the number of these increases and similar ones make their appearance in the next younger section of the stele, that belonging to the fourth leaf. Last of all, these primary tracheids are formed in the younger region of the stele and new ones arise between these first-formed ones, so that there is developed a nearly complete circle of tracheids marking the inner boundary of the tubular stele.

The second leaf trace approaches nearer and nearer the two horns of the crescentic stele section as it is followed downward, and finally joins it, filling up the gap between the regions belonging to the third and fifth leaf traces. Thus, for a

time the section of the stele appears perfectly circular and continues downward until it reaches the next leaf trace, which marks the point of departure of the trace of the cotyledon. Except for the greater development of the xylem, which is composed of two to three concentric rows of tracheids, the section of the stele in this region presents much the same appearance that it does in the younger parts nearer the stem apex. The outer part of the stele at this point shows a somewhat broken row of thick-walled bast fibers within which are large sieve tubes. The cambium, lying just inside the phloem, is very much less developed in the basal region of the stele than it is higher up and can scarcely be said to be present. The stele in this region of the stem is quite similar to that of *B. lunaria*, figured by Poirault. The endodermis is also much less definite than it is later, and I could not satisfy myself that there was in *B. virginianum* any trace of the inner endodermis which Poirault states is present in the basal part of the stem in *B. lunaria*, but which disappears later. The medullary rays also, which are conspicuous in the stele of the older plant, are very imperfectly developed in this basal region.

In the intermediate region, between the base of the primary root and the trace from the cotyledon, a section shows a thick irregular ring of tracheary tissue with no clearly developed medullary rays (fig. 43, *L*). Indeed, at the lowest part of this region the tracheary tissue forms an almost solid core with only a small amount of parenchyma interspersed, thus forming an inconspicuous and irregular pith. While the endodermis is not clearly delimited, some of the cells in the zone of tissue surrounding the stele show the radial thickenings of the walls; but these cells are not all of them in the layer immediately adjoining the stele, being irregularly disposed throughout the three or four layers of cells surrounding the stele. As sections are examined in succession upwards, the pith becomes better defined. Sections taken downward show a ring of tracheary tissue gradually separating into the two or three xylem masses of the primary root.

The primary root in the sporophyte which has just been described was diarch. The stele shows a very evident endodermis, within which is the pericycle, for the most part composed of two layers of cells. The xylems are composed of a large group of tracheids, of which the two or three smaller ones next the pericycle represent the protoxylems. Small, deeply staining protophloem elements can be seen toward the outside of the phloem, and within these are the other phloem elements, thick-walled bast fibers, and sieve tubes. The rest of the stele is composed of small, thin-walled parenchyma cells. The outer region of the root is made up of somewhat compressed cells suggesting the periderm cells which are found in parts of the older stem and which are also present in the cortex of the later roots. This outer layer of cells, however, shows no evidence of active division.

A cross-section of the petiole of the cotyledon, from the same sporophyte, taken at a point some distance above the stipules, shows within the epidermis about three layers of parenchyma cells with no conspicuous intercellular spaces, but within these are several very large lacunæ, separated by narrow plates of tissue. In the center of the section are seen the two concentric bundles that traverse the petiole. As in the later leaves of this species, these bundles are truly concentric, thus resembling those of the Marattiaceæ and differing from the collateral bundles of *Ophioglossum* and the smaller species of *Botrychium*. The phloem is better developed upon the outer side and small protophloem elements can be seen at its outer limit, but these are continued partially around the inner side of the bundle, where the xylem is separated from the outside by about two layers of cells. No definite endodermis can be made out. The two bundles are continued up to the base of the lamina, where one of them passes into each lateral lobe of the ternate leaf. One of

these divides and gives off a branch which passes into the terminal lobe of the leaf (see Jeffrey 1, page 23).

In a section of the older stem, about where the fourth leaf trace becomes joined to the fifth and sixth, the section of the stele appears circular. In the portion belonging to the fifth leaf trace there are present about half a dozen isolated tracheids, arranged in a row, while in the sixth trace only a single small tracheid is developed. The two xylems of the fourth leaf trace are now no longer distinguishable and the woody part of the trace has the form of an irregular row on the inner side of the trace section, and is continuous with the xylems of the fourth and fifth traces. Even after the fusion of these three traces is practically complete, it is still evident that the ring-shaped section is composed of three parts, the break between the xylems showing the limits of the three component leaf traces. With the increase in the development of the xylem ring the limits of the individual leaf traces are finally completely lost and the section of the stele shows a thick ring of the tracheary tissue interrupted only at intervals by single rows of parenchyma cells in the medullary rays.

Outside the ring of tracheids there can be seen a zone of narrow cells arranged in radially disposed rows. This zone constitutes a genuine cambium, precisely similar in appearance to that found in the stems of Conifers and Dicotyledons. This cambium has long been known to occur in the older sporophytes of the larger species of *Botrychium*, and Jeffrey first noted it in the young sporophyte. The cambium is well developed in the leaf trace for a considerable distance above its junction with the central stele.

The primary tracheary tissue is in immediate contact with the pith. Occasionally the ring of tracheids is broken by a single parenchyma cell. Outside the tracheary ring the cells are arranged in radial rows and in these the formation of the walls is always periclinal, so that the cambium in these earlier stages presents a very characteristic appearance. The rows of cambium cells continuous with the primary parenchyma cells lying between the tracheids of the primary row do not develop tracheary tissue, but remain parenchymatous and thus give rise to the medullary rays, which later are so conspicuous.

The extreme outer portion of the vascular cylinder is composed of a ring of thick-walled cells which are probably bast fibers, but the walls of these do not stain strongly with safranine and are hence much less conspicuous than the tracheids. These may possibly be considered as protophloem elements and evidently correspond to the zone of thick-walled cells figured by Poirault for *B. lunaria* (Poirault 2, fig. 11). Just outside of this ring of thick-walled cells lies the endodermis, which has not as yet developed the characteristic radial thickenings which are so easily seen in the older parts of the stele. These thick-walled phloem elements, as is the case with the protoxylems, do not form an unbroken ring, but are interrupted at the junctions of the confluent leaf traces (fig. 44, *C*).

In the older bundle, a zone of large cells, the young sieve tubes may be recognized lying inside the ring of bast fibers. Inside of the zone of sieve tubes lies the cambium, the cells of which gradually pass into the xylem, to which constant but slow additions are made from the cambium; indeed, Jeffrey thinks that we can not speak of primary wood. He considers that all of the wood owes its origin to the activity of the cambium ring. The radial rows of secondary wood are interrupted at intervals by the medullary rays, and the resemblance of the stele at this stage to the section of a young coniferous stem is really quite remarkable. The endodermis has now become exceedingly conspicuous, as the lignified lateral walls stain very strongly with safranine and stand out strongly in contrast with the cells of the cortex lying outside the endodermis. The inner walls of the endodermal

5

cells also stain more or less strongly with the safranine, indicating that they also are partially lignified.

The junction of the second leaf trace with the central stele is shown in fig. 43, *E*, *F*, *G*, and similar stages in the fusion of the first leaf trace are shown in fig. 43, *H*, *I*. The junction of one of the early roots, perhaps the third root, with the central stele is shown in fig. 43, *I*. At the level where the second leaf trace departs from the central stele the xylem ring is composed of about three rows of cells. In both this second leaf trace and the third the endodermis is very conspicuous upon the outer side of the bundle, but is not developed upon its inner face. As the trace approaches the central cylinder it can be seen that the endodermis is interrupted across the leaf gap, but as the leaf trace approaches, endodermal cells are developed between the endodermis of the central cylinder and that of the leaf trace. This union takes place first upon one side, the gap being closed sooner on one side than the other, but later a similar process takes place on the other side of the leaf trace and the closing of the gap is complete, the endodermis now being continued without interruption around the whole of the circular section. The leaf trace where it joins the central cylinder is exactly like it in size and structure; indeed, it is nothing more than a sector of the hollow cylindrical stele, which is evidently built up exclusively of these fused leaf traces. There is no vestige of *any* tissue in the stele which is not referable to the bases of the leaf traces themselves. This becomes very evident when the structure of the leaf trace is examined before it closes the gap where it joins the central stele.

An examination of the outer tissues of the older portions of the stem (fig. 42) show that at certain points there is a conspicuous border of periderm (*per*), forming a layer some four or five cells deep. This border of periderm occupies about a third of the circumference of the section and marks the position of an old leaf base which has fallen away. According to Jeffrey, this periderm is an "abciss" layer and results in the cutting off of the old leaf bases. He suggests that the layer of cork cells developed from the periderm closes the scar left by the dead leaf and may be efficacious in preventing infection from fungi in the soil. The presence of a periderm in the outer cortex of the Ophioglossaceæ was first pointed out by Russow (Russow 1), and was afterward confirmed by Holle (Holle 1). The periderm in *B. virginianum* is made up of the usual radially arranged rows of cells (fig. 42, *per*).

All of the tracheary tissue in the young sporophyte is composed of rather short, reticulately marked tracheids. The secondary tracheids increase in size and the thickened bars on their walls become broader, so that the larger elements have their walls pitted rather than reticulately marked, and these pits, especially in the older plant, are of the bordered type, not very unlike the round bordered pits found in the wood of Conifers.

The elongated thick-walled elements in the outer part of the phloem are probably bast fibers. In the older part of the bundle these stain quite strongly with safranine and show irregular pits upon their walls. The sieve tubes do not show at all clearly in sections mounted in balsam. They are evidently large, but the characteristic dotted sieve areas can not be made out and no special study was made of these. Between the large secondary tracheids and the phloem are the narrow, thin-walled cambium cells. These probably contribute only to the wood, and there is no secondary phloem.

### THE YOUNG SPOROPHYTE OF HELMINTHOSTACHYS.

The only account that has yet been published of the young sporophyte of *Helminthostachys* is that of Lang (Lang 1), who examined at some length the vascular system in the young sporophyte. Lang states that the young sporophyte of *Helminthostachys* develops its first leaf as a large ternate foliage leaf, and when I first examined the young plants which were collected in Ceylon I reached the same conclusion; but on making sections of the young sporophyte it was found that in all of the young plants that were sectioned the supposed cotyledon was really the second leaf, the cotyledon itself being rudimentary and forming an inconspicuous papilla at the base of the second leaf, which was the first to develop a functional lamina. Further examination of a number of other specimens showed that this rudimentary cotyledon was also present and it is probable that the cotyledon in *Helminthostachys*, as in *Botrychium lunaria* and *Ophioglossum vulgatum*, is always a rudimentary organ which never appears above the ground.

The youngest sporophytes of *Helminthostachys* which were found already had the second leaf well developed and the first root had emerged. The ternate second leaf (fig. 10, *C*) shows clearly that the ternate form is the result of an unequal dichotomy, such as is common in the early leaves of many ferns. The second leaf of *Helminthostachys* closely resembles the cotyledon of *Botrychium virginianum* and, like that, usually possesses two vascular bundles extending through the long, slender petiole. At the base of the leaf there is a conspicuous stipular sheath which completely incloses the next younger leaf, as it does in *Botrychium lunaria*. The two bundles of the petiole contribute to the two lateral lobes of the leaf as they do in the cotyledon of *Botrychium virginianum*, and one of these, forking again, gives rise to the terminal lobe and smaller lateral lobe. The venation of the second leaf, especially in its younger stages, is almost perfectly dichotomous and is more like the venation in the leaves of *Botrychium lunaria* than that of *B. virginianum*. With the enlargement of the leaf segments the venation more nearly approaches the pinnate arrangement found in the later leaves.

While the young sporophyte in *Helminthostachys* in its earliest stages shows a general resemblance to that of *Botrychium virginianum*, a difference is very soon noted. The young plant, very soon after the formation of the cotyledon, develops a conspicuous internode between the cotyledon and the root, and the stem rapidly elongates as the sporophyte grows, so that it soon shows the characteristic elongated rhizome of the older sporophyte.

Unfortunately I was unable to secure any very young sporophytes, my youngest specimens already having the second leaf nearly fully developed. According to Lang, the arrangement of the primary organs of the young sporophyte is the same as in *B. virginianum*, but I could make no study of the origin of the vascular system in the sporophyte before the first leaf and root had emerged from the prothallium.

To judge from longitudinal sections of the youngest plants obtained, the relation of the first leaf and root is the same as in *Botrychium virginianum* and the base of the young sporophyte closely resembles that of the latter. As in *Botrychium*, the foot is very large and conspicuous, forming a large, hemispherical body surrounded by the prothallial tissue. The large primary root grows out at right angles to the foot and is continued into the axis of the sporophyte, which grows up vertically. None of the specimens were young enough to show the relation of the stem apex to the first leaf and root, as in the youngest specimens the axis had already begun to elongate and an evident internode was formed between the base of the first leaf and the root.

The longitudinal section of the young sporophyte at this time shows that the thick stele of the root bends upward and is continued into the axis, which already is a good deal elongated and has carried up the young leaves, which are separated from each other by conspicuous internodes. The young sporophyte at this stage thus differs strikingly from the corresponding stages in *Ophioglossum* and *Botrychium*, with their very closely crowded leaves with no appreciable internodes between them (fig. 46).

A single stele extends through the axis from its junction with the root stele and forks below the insertion of the rudimentary first leaf, one branch passing into the cotyledon, the other continuing upward and forming the trace for the second leaf, at whose base is seated the apical bud.

Whether in the younger stages, before the development of the second leaf, the vascular system of the young sporophyte would show the same relations of the steles of the root and cotyledon, as in *Botrychium*, remains to be seen; but from the general similarity in the structure of the young sporophytes of the two this is quite likely: yet it is possible that the stem apex is more prominent in the early stages of *Helminthostachys* and part of the stele which is found in the elongated stem may be of cauline origin, although this is hardly indicated by the condition of things in the apical meristem of the youngest sporophytes that were examined.

The foot is composed of large-celled, thin-walled parenchyma, with no noticeable differences between the inner tissue and that of the periphery. The stele of the root is continued upward without interruption into that of the shoot (fig. 46, *B*). Probably at an earlier period there would have been, as in *Botrychium*, simply the continuous stele of the root and cotyledon. At the junction of the root stele with that of the axis there is a marked enlargement, and the short, somewhat irregular tracheids are decidedly broader than those either in the root or in the portion of the stele above the junction. The tracheids toward the outside of the central region are slender and have delicate reticulations, which closely approximate the form of true spiral thickenings. These are the protoxylem elements and can be traced upward and downward into the stem and root, respectively. The secondary tracheary tissue is marked by broad, reticulate thickenings, and these in the largest tracheids are replaced by conspicuous oval or nearly round bordered pits, very much like those which are found in *Botrychium* (fig. 46, *E*).



FIG. 45.—A. Young sporophyte of *Helminthostachys*, attached to gametophyte, *pr. rot*, cotyledon; $r^1$, $r^2$, first and second roots. × 2. B. Leaf from an older sporophyte. × 1.

Above the base of the root there is a long internode below the cotyledon, and through this runs the single axial stele. On the same side of the axis as the primary root may be seen the cotyledon, which closely resembles in form the young cotyledon of *Botrychium virginianum*, but instead of developing into a functional leaf it is arrested in its growth before the lamina is fully developed. As in the cotyledon of

*Botrychium virginianum*, the lamina, which although very greatly reduced nevertheless is present, is bent over sharply against the stout petiole, which is strongly convex on its adaxial side. In the specimen figured there was no trace of a stipular sheath developed in the cotyledon, but sometimes this is very well developed, although the upper part of the cotyledon remains quite rudimentary. The little cotyledon has the rudimentary lamina distinctly ternate in outline and very much resembles some of the rudimentary early leaves found in *Botrychium lunaria*, as figured by Bruchmann. A single vascular bundle extends through the petiole of the cotyledon, nearly to the base of the lamina, but except in the basal region the development of the xylem is much reduced and is entirely lacking before the lamina is reached. The bundle of the cotyledon, where it bends at the base into the internode, is nearly as well developed as the bundle of the second internode which separates from the former just below the insertion of the cotyledon. On the opposite side of the second internode from the cotyledon, and extending downward through the first internode for a short distance below the level of the cotyledon, there is seen
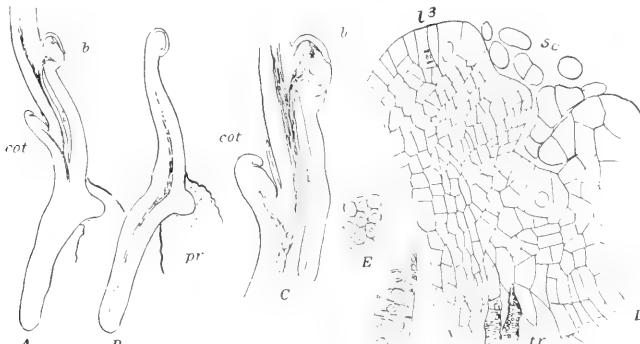


Fig. 46.

A, B. Two longitudinal sections of a young sporophyte of *Helminthostachys*; *pr*, gametophyte. ×6.
C. Upper part of same, more enlarged; *b*, terminal bud; *cot*, cotyledon; *i*, lacuna in first internode.
D. Apical region. *l³*, third leaf; *sc*, epidermal scales. ×140.
E. Tracheids showing bordered pits and thickened bars. ×320.

in the specimen figured a large lacuna, separated from the outside of the internode by about half a dozen layers of cells and on its inner side from the endodermis of the stele by two or three layers of cells. This lacuna, as Lang pointed out, is not always present. At the upper end of the second internode can be seen the base of the second leaf, the stipular sheath of which incloses the young bud at the summit of the axis.

On its inner (dorsal) side the terminal bud shows the young third leaf, opposite which is the section of the ridge surrounding the small free surface of the stem apex, very much like the condition in the young bud of *Ophioglossum moluccanum*. The form of the young leaf at this stage is more like that of *Botrychium lunaria* than that of *B. virginianum*. The young leaf is a blunt cone with a marked projection on its inner side, extending over the apex of the stem and marking the beginning of the stipular sheath, which is entire as in *B. lunaria*, and not divided into two free stipules as in *B. virginianum* (fig. 46, *D*). A conspicuous strand of procambium extends into the leaf and joins the trace of the second leaf. As in *Botrychium* and *Ophioglossum*, the stele of the internode does not extend upward beyond the base of the youngest leaf and there is no indication that it derives any of its tissues directly

from the apical meristem of the shoot. The latter, as in the other Ophioglossaceæ, shows a large single initial cell, which in section closely resembles that of *Ophioglossum moluccanum*, having a broadly truncate base and being narrower above (fig. 46, *D*). The apical meristem is of very limited extent and segmentation of the apical cell is probably very slow. The second root, which in the case under consideration arose near the base of the third leaf, is here very conspicuous and its stele joins that of the third leaf near the base of the internode. The young leaf and root, with the inconspicuous stem between them, have very much the appearance of a bud at the base of the second leaf. The apex of the very young leaf grows from the conspicuous single apical cell (fig. 46, *D*). This appears triangular in longitudinal section and shows regular segmentation. The stele of the young leaf can be traced up to the inner cells, cut off from the lateral segments of the apical cell.

The apical cell of the stem is quite large and, as we have seen, is truncate below, as it is in *Ophioglossum*. Seen in its transverse section it is triangular, so that its form is really that of a three-sided prism. Both lateral and basal segments are cut off from it, but only the youngest segments can be clearly distinguished. Below the apical cell there is only a very small amount of tissue between the apex of the shoot and the point of the junction of the two leaf traces and the stele of the second root. This junction is marked by the presence of very irregular reticulate tracheids (fig. 46, *D*, *tr*). The tissues on the upper side of the root stele extend to within a few cells of the stem apex and may possibly represent a portion of the internodal stele that is not connected with the leaf trace, but a study of later stages does not support this view, and it is almost certain that this youngest tracheary tissue is the beginning of the next leaf trace.

From the very first, as Lang pointed out, the shoot is perfectly dorsiventral, although at first it grows vertically upward. The leaves are all borne upon the dorsal side, while the roots invariably arise upon the ventral side.

The apex of the young root, which in this case is on the point of emerging, is occupied by a conspicuous triangular apical cell, the segmentation of which closely resembles that
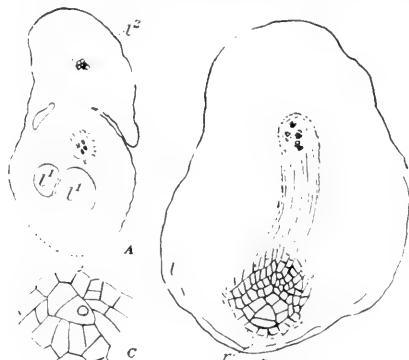


Fig. 47.—Transverse section of young sporophyte of *Helminthostachys*, above terminal bud. $l^1$, $l^1$, lacunæ; $l^2$, second leaf. ×65.

B. Section passing through base of bud. *r*, young root. ×100.
C. Stem apex. ×200.

of the root of *Botrychium*. At first the root cap is mainly derived directly from the outer segments of the apical cell. These segments undergo periclinal as well as anticlinal divisions and the inner layers of the root cap (which at this stage is relatively thick) show a fairly regular stratification. The outer cells of the root cap are a good deal elongated, so that the free end of the root is decidedly pointed. At this time, except for the irregular conjunctive tracheids at the base, no permanent tissue can be seen in the stele of the young root.

In the cross-sections of a young sporophyte of about the same age the third leaf was rather further developed and the young fourth leaf could be seen, but the second root was not so far advanced, or it may be that the root at the base of the third leaf was the third root and not the second. The single apical cell, which was so prominent

in the very young leaf, can no longer be recognized and the lamina is divided into two nearly equal lobes, of which one is lateral with reference to the other, but these two lobes probably arise as a result of the dichotomy of the original apex. A group of narrow marginal cells somewhat to one side of the terminal lobe evidently constituted a meristem tissue, and this was preparing for the second dichotomy, by which the ternate form of the young leaf is established. The branching of the lamina at this early period therefore appears to be the result of an unequal dichotomy, as it is in the first foliage leaf. The vascular bundles from each of the two primary leaf segments were just beginning to be recognizable and join into one at the base of the lamina. Surrounding the leaf is the sheath developed from the base of the second leaf. This sheath in the section appears as a closed ring some two or three cells in thickness and looking very much like the sheath of *Ophioglossum*. The top of the conical sheath is closed and does not show the pore found in *Ophioglossum*. The sheath becomes thicker lower down, where four rows of cells show in the section. Between the inclosed leaf and the surrounding sheath in the lower portion (fig. 49, *B*) may be seen the section of flattened scales (*sc*) which grow from the base of the leaf
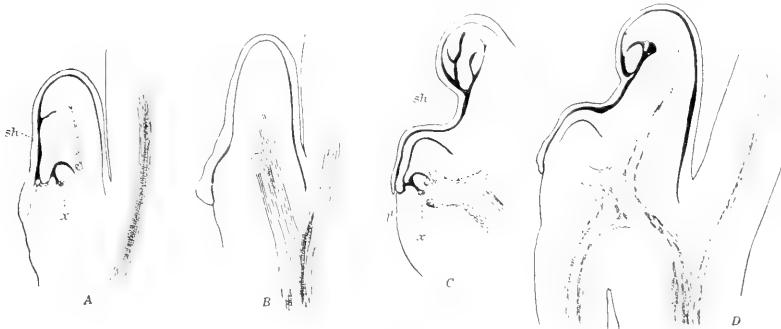


FIG. 48.

A, B. Two sections of terminal bud of a young sporophyte of *Helminthostachys*.
C, D. Two similar sections from an older plant. *x*, stem apex; *sh*, petiolar sheath.

and from the tissue immediately about the stem apex. At its base the sheath of the third leaf is already completely developed and surrounds the young fourth leaf ($l^4$), which in this section was cut horizontally and shows plainly its large apical cell. The stem apex, which lies directly below the younger leaf, was cut obliquely in this series and did not show the form of the apical cell plainly, but in a young plant of about the same age (fig. 47, *C*) the apical cell showed that it was regularly triangular in form, seen in cross-section.

At the level of the stem apex the second leaf shows a single vascular bundle which may be continued undivided upward, or it may divide into two bundles in the petiole. In regard to the structure of the petiole, therefore, *Helminthostachys* is to some degree intermediate between *Botrychium* and *Ophioglossum*. The young trace of the third leaf can be seen at this level as an oval mass of undifferentiated cambium. Immediately below the stem apex the cells are smaller than is the case in the young sporophyte of *Botrychium*, but one can not make out the beginning of a central procambium cylinder. The smaller cells in the region immediately underlying the stem apex may be explained by the fact that in these young plants there is no pith developed within the axial stele as there is later on. The youngest root in this

specimen was less developed than that in the specimen that we have just described. This youngest root was cut obliquely, but at its base there could be seen the undifferentiated mass of procambium forming its stele, and the apex showed the tetrahedral apical cell, presenting much the same form as the longitudinal section. The apical portion of the young root was somewhat shrunken away from the surrounding tissue, so that there was a very evident space surrounding it. Whether or not this was the result of artificial shrinkage was not certain.

Below the terminal bud the third leaf trace proceeds downward into the internode below, and the tracheary tissue, which is just beginning to develop in the bud region, increases rapidly in amount and forms a central mass of wood which does
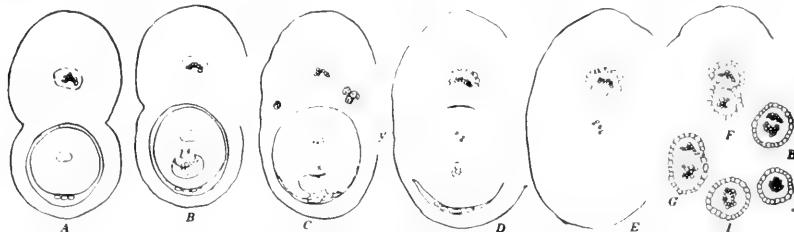


Fig. 49.—Ten cross-sections from a series of a young sporophyte of *Helminthostachys*. ×25.

C. Passes through the stem apex, *x*. The round shaded cells are tannin sacs.
D. Shows the narrow pit, *y*, between the leaf base and the axis.
G–J. Show only the vascular bundles.

not appear solid, but is made up of two groups of tracheids, of which the one nearest the second leaf trace has the larger tracheids. At this stage the bundle somewhat resembles the section of a diarch root. Only a little further down the two xylem masses are joined by the development of tracheids in the middle of the bundle, which thus comes to have a solid xylem core. In the section figured there may be seen a single small isolated tracheid on the side turned toward the second leaf trace, which has now approached so close to the central bundle as to touch it (fig. 50, *A*). The central bundle in the internode at this point is beyond question the leaf trace for the third leaf. There is no difficulty in tracing its progress upward to where it enters the leaf and there is no sign of any addition to it from tissue lying outside the original leaf trace. The central stele of the upper part of the second internode is throughout nothing more than the basal portion of the leaf trace from the third leaf. At this point it is concentric in structure. Surrounding the solid xylem core can be seen about three rows of phloem cells, some of the outer ones being small protophloem elements. The large ones lying inside are presumably sieve tubes. A single layer of rather large pericycle cells separates the phloem from the large endodermal cells, which begin to show very conspicuously lignified radial thickenings, especially on the side of the bundle adjacent to the second leaf trace. The section of the second leaf trace is somewhat extended transversely and the xylem band is composed of about a dozen tracheids. In the mid region of the section the xylem is separated from the inner side of the bundle by a single layer of cells, possibly sieve tubes, but at one point the xylem is in contact with the pericycle, which is only imperfectly developed and wanting at certain points. Upon the dorsal side the phloem may be in direct contact with the endodermis, which is quite wanting at this point upon the ventral side of the bundle. Higher up, where the trace is quite free, the endodermis extends completely around it. While the second leaf trace, therefore, may

be said to be concentric, it nevertheless approaches closely the collateral type of the other Ophioglossaceæ.

The fusion of the two leaf traces to form the single axial bundle in the basal part of the internode is very much like that found in the young sporophyte of *Botrychium* (fig. 49, *D, F*). The endodermis of the two bundles becomes continuous and there may be seen intermediate stages of a single bundle, oval in section, but with two conspicuous xylems, separated by the mass of large, thin-walled tissue which gradually disappears as the bundle is followed downward; and finally, in the lower part of the internode, the section of the stele appears quite circular, the center being occupied by the solid xylem formed by the complete coalescence of the xylems of the fused leaf traces (fig. 49, *J*). The arrangement of the phloem, pericycle, and

endodermis is the same as in the single leaf trace in the upper region of the internode.

The base of the stipular sheath can be followed downward to about the level where the two leaf traces begin to coalesce. Its anterior free portion, between which and the internode is a narrow slit, finally becomes quite free and its section is visible as an oblong mass of cells lying quite separate from the section of the internode.



FIG. 50.

Two sections of the vascular bundles from the series of *Helminthostachys* shown in fig. 49, showing junction of two leaf traces to form central stele. *en*, endodermis. ×150.

Longitudinal sections of a somewhat older sporophyte are shown in fig. 48, *A, B;* the third leaf is pretty well developed, with the stipular sheath completely overarching the stem apex and the fourth leaf, which already is conspicuous. The third leaf is still completely covered by the large stipular sheath of the second leaf, which apparently forms a closed cavity; but an examination of the adjoining sections showed that the anterior margin of the sheath is quite free in front and that there is a narrow cleft between it and the internode. The real nature of the sheath and its relation to the leaf base is very clearly shown in the median section of the terminal bud from an older plant (fig. 48, *C, D*), and the resemblance to the terminal bud of *Botrychium lunaria*, except for the dorsiventral arrangement of the parts, is most striking. This is equally marked in the adult sporophyte, as Farmer has already pointed out.

The specimen under consideration differed somewhat from the younger one that was described in the relation of the young organs. The cotyledon was better developed and although the lamina was rudimentary the stipular sheath was large and inclosed the base of the second leaf, while in the younger specimen discussed no sheath was developed at the base of the cotyledon. There was a different relation of the roots also, probably associated with the greater development of the cotyledon in the specimen under consideration. The second root, instead of being formed at the base of the third leaf in the terminal bud, was developed a short distance above the cotyledon, near the base of the second leaf, while in the younger specimen no root was developed above the primary root until the third leaf had
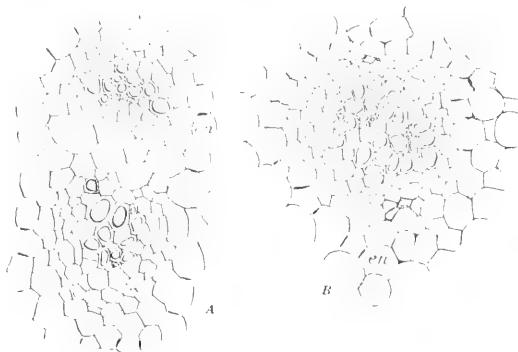
begun to develop; but this second root developed much earlier in its relation to the development of the next leaf than was usually the case.

In the present plant the root corresponding to the third leaf, which was the third root in this case, was in a very early stage of development and had not begun to elongate at all. The third leaf in median section was already pretty well advanced and the apical portion was very strongly incurved, as it is in the young leaves of *Botrychium virginianum*, which it resembles more than it does that of *B. lunaria*, and it also suggests the form of the young leaves in the Marattiaceæ. The basal part of the young leaf, however, is exactly like that of *B. lunaria*. The stipular sheath forms a thick body, projecting forward and about equal in height to half the total length of the young leaf. It is strongly concave below, and the next leaf is fitted into the cavity (fig. 48, *C*). The forward margin extends downward as a sort of lip which elongates rapidly and keeps pace with the growth of the young leaf inclosed within it, and this is completely concealed until it has reached a large size. The sheath finally forms a long, conical protuberance at the base of the leaf to which it belongs.

The cavity below the sheath is extended backward, so that the base of the leaf is hollowed out in front and in section appears much narrower than the part of the petiole above the insertion of the sheath; this brings the stele of the leaf very close to the epidermis at this point. The narrow cleft thus formed between the posterior wall of the stipular cavity and the petiole of the leaf in front is the "canal," which Gwynne-Vaughn described as occurring at the base of the petiole of the older plant and opening above the insertion of the stipule. These canals are easily seen in longitudinal sections of the young bud (fig. 48, *B*) and it is very clear that they are, in truth, nothing but the narrow spaces between the bases of the adjacent leaves. Gwynne-Vaughn's statement that they open above the insertion of the stipules is incorrect unless the stipules of a given leaf are considered to be derived from the sheath surrounding the base of the leaf, this sheath properly belonging to the next older leaf. This canal can be seen as a narrow slit in the young leaf, extending below the insertion of the next younger one, the young stele of the leaf being separated from the epidermis at this point by only about three rows of cells.

The stele of the young leaf can be traced nearly to its tip, and then continues downward toward the inner side of the leaf into the internode, below where it joins the young stele belonging to the next younger leaf.

As in the younger sporophyte, the stem apex is of very limited extent and the apical cell shows the same form. In the section figured (fig. 48, *C*) the youngest leaf is cut almost in a median plane and though externally no differentiation is visible the young stele is already conspicuous and can be followed down without difficulty. Passing on one side of the stem apex and above the leaf trace, there is no evidence of procambium in the apical region of the stem. In the young sporophyte, therefore, it is perfectly certain that no cauline stele is present. Farmer (Farmer **2**) states that in the older sporophyte the stele can be traced above the insertion of the youngest leaves, but we believe a careful study of this point in the older plant would show, as in the younger one, that the stele is developed in the young leaf at an exceedingly early period and that the stele of the internode is composed entirely of leaf and root traces. The procambium tissue developed on the ventral side of the stele near the apex can be shown, by a study of its transverse sections, to be due entirely to tissue derived from the leaf traces, which are extended downward until they meet on the ventral side of the internode.

Near the stem apex are numerous small scales and hairs filling up the space about it and probably associated with the prevention of drying up of the stem

apex, which is doubly protected by these scales and its complete inclosure in the young leaf bases. It is possible that these scales secrete some mucilaginous substance, although there was no evidence of this in the stained sections.

In this plant the internodes were quite solid and there was no trace of the large lacunæ which were described for the younger plant. Whether the presence of the lacunæ is due to any differences in the environment remains to be seen. The specimens were all collected under apparently the same conditions, but it is possible that there may have been differences in the amount of water in the soil in which they were growing, and this difference in the amount of water may have something to do with the development of the lacunæ.

The root at the base of the third leaf was cut transversely and was in a very early stage of development. The root was still completely buried in the cortex and the stele still imperfectly developed, but showing its connection with the stele of the fourth leaf above the junction of the third leaf trace with the stele of the internode. No tracheary tissue was developed and the course of the leaf trace was but little disturbed by the formation of the root.

In longitudinal sections taken from a much older sporophyte (fig. 48, $C$, $D$) the arrangement of the organs at the apex is seen to be exactly the same as in the younger plants, but of course the parts were all larger. The youngest leaf ($l^1$) still resembles in form and size the corresponding leaf in the younger stem, but the next older leaf is relatively broader than the early leaves of the younger sporophyte, and although the stipular sheath is now well developed the upper portion of the leaf is still quite undifferentiated and the apex is scarcely bent forward at all. The resemblance, therefore, to the young leaf of *Botrychium lunaria* is even more marked than it is in the younger sporophyte. The apical region of this young leaf is occupied by an epithelium-like layer of columnar cells, and it is doubtful whether any one of these can be certainly denominated the apical cell. Somewhat the same doubt exists also as to the point of a single initial cell in the youngest leaf. In the latter there was some shrinkage in the group of large meristem cells at the apex, which made it still more difficult to decide this point, but a median section showed somewhat indistinctly a single cell, apparently triangular in outline, which from its form and the arrangement of the adjacent cells may very well have been the apical cell. The small group of meristem cells forming the stem apex is crowded in between the base of the youngest leaf and the elongated ridge which surrounds the stem apex upon the ventral side. In consequence of this crowding the outer faces of the apical cell and its youngest segments are very small. The base of the apical cell is more than twice as broad as its free outer face. The ventral face of the apical cell is convex and the young segments cut off from it are strongly curved. The inner or basal wall is oblique, so that the axis of the young cells in the tissue below the apical cell makes an angle with the long axis of the apical cell.

Below the apical region may be seen the section of the large central stele of the internode. This is composed, apparently, of two strands of procambium separated by a broad band of pith. As Farmer pointed out, in the older plant there is no question that procambium tissue is developed upon the ventral side of the bundle which extends into the apical region and which is, apparently, not connected with the leaf traces. From this he believes that the stele is really a cauline structure.

A study of the cross-sections, however, as will be presently seen, shows that this ventral tissue really does belong to the leaf traces, although it is possible that the basal tissue of the roots may add to it in part, but I have not been able to satisfy myself that any of this stelar tissue can be properly assigned to the activity of the stem apex.

As in *Botrychium* and *Ophioglossum*, the tissue derived immediately from the activity of the apical meristem, after the central pith is developed in the stele, contributes only to this central region or pith, which is really not part of the stele proper. On the dorsal side of the stele the whole of the procambial strand can easily be followed into the leaf, and when this is followed downward it is seen to run obliquely through the youngest internode, at the base of which it joins the next leaf. The latter already has its lamina developed and strongly bent over, but is still inclosed in the stipular sheath belonging to the next older leaf.

These older leaves have only a single leaf trace, but it divides into two strands above the level of the stipular sheath. A median section of the thick central stele in
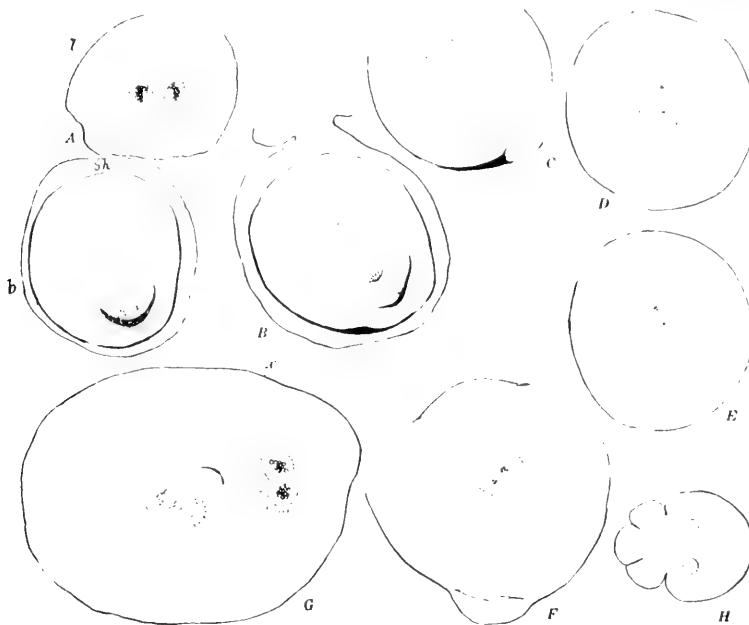


FIG. 51.

Seven transverse sections from a young sporophyte of *Helminthostachys*. ×35. *l*, petiole of leaf; *b*, terminal bud inclosed by stipular sheath, *sh*. In G, *x* is pit between leaf base and internode. H, section of a young leaf, showing the ternate lamina.

the upper region shows conspicuous protoxylem elements made up of much elongated tracheids, whose thickenings are between annular and spiral in form, but very different from the coarsely reticulate and the conspicuously pitted tracheids of the metaxylem developed in the older parts of the stele. In all cases where truly median sections were seen the protoxylem elements occupied the innermost region of the xylem and no metaxylem was seen within these, so that the bundle (at first, at any rate) is endarch, as in the other Ophioglossaceæ. It is impossible to see in this section that metaxylem was developed inside of the protoxylem, as Farmer found to be the case in the older sporophytes.

In fig. 48, *D*, there is shown an insertion of a root upon the stele and its very broadly expanded base with the irregular conjunctive tracheids. This very broad base of insertion and the obvious continuity of the tracheary tissue of the root with that of the central stele of the stem suggest that the ventral part of the stele might be made up, in part at least, of root traces.

Cross-sections of older plants, such as that shown in the series indicated in fig. 51, show essentially the same arrangement of the parts as do the younger ones. At the apex there may be seen in section the three youngest leaves, which we will designate respectively 1, 2, and 3, No. 1 being the oldest leaf shown. In this section the oldest leaf shows two vascular bundles in the petiole, and this is true also of the second leaf, which is contained within the stipular sheath of the oldest leaf. The lamina of leaf No. 1 already shows the characteristic ternate form. The young leaves make an angle of about 30° with each other. Section *B* passes directly through the stem apex and shows the sections of the basal part of the sheath of the two older leaves. The stipular sheath of the youngest leaf partially incloses the stem apex, and surrounding the apical region is a loose mass of cells, derived partly from the tissue immediately around the stem apex and partly from the edges of the sheath of the youngest leaves. These cells are really sections of hairs and scales which perhaps secrete mucilaginous matter, but there was no evidence of this in the sections. Turned toward the dorsal side of the section may be seen the leaf trace from the second leaf and separated from it by a considerable space on the ventral side is a rather vaguely defined mass of young procambium, which marks the trace of the youngest leaf. On the ventral side of the latter is the stem apex itself. Further down, as has already been described for the younger stem, the two leaf traces approach. At the level of the stem apex the trace of the second leaf is clearly defined and nearly oval in outline, but more convex upon the dorsal side. The bundle is inclined toward the side of the stem opposite the insertion of the first leaf. Further down the trace becomes much broader, this being mainly due to the development of the tissue upon one side, which begins to bend downward toward the ventral side of the stem. The trace of the youngest leaf is now somewhat better defined and appears somewhat kidney-shaped in section, the convexity, like that of the second leaf trace, being turned toward the dorsal side. This trace also begins to show the extension of tissue toward the ventral side of the stem and this is developed on the side opposite to the extension in the next older leaf trace. Ultimately these ventral extensions of the two bundles meet as the two leaf traces come nearer together, and the dorsal ends also finally come into contact, so that the two bundles, the section of each of which is approximately semicircular, form a complete ring inclosing a greater or less amount of the ground tissue, which thus forms the pith of the hollow stele. The stele resulting from the union of the two leaf traces is not at first perfectly circular in section, but shows plainly for a long time that it is composed of two separate bundles (fig. 51, *F*).

The first development of permanent tissue in the older leaf trace is evident before it joins the younger one. The first elements are thick-walled cells (which may be called protophloem) in the outer zone of the phloem, and a little later at the inner limit of the bundle a small group of protoxylem elements appears. Very soon after this a similar differentiation takes place in the younger leaf trace, so that when the two leaf traces are completely fused the protoxylems have the appearance of having arisen toward the central part of the bundle, but in reality the component bundles, at least at their earlier stage of development, may be described as endarch and agree, therefore, with the bundles of the other Ophioglossaceæ. In the leaf traces of the older part of the stem, however, there may generally be found inside

the protoxylem a few scattered, large tracheary elements, so that the bundle may be said to be mesarch, as Farmer states is the case in the older rhizome.

During its earlier stages the stele shows no leaf gaps where the leaf traces depart, and the leaf gaps are only gradually developed. Soon after the stele has assumed the form of a hollow cylinder the leaf gaps are for some time absent, or they are developed only in a very small degree and close almost immediately upon the departure of the leaf trace.

In none of the young plants that I examined could I detect any of the inner endodermis which occurs in the older rhizomes. Farmer, however, states that the inner endodermis is only imperfectly developed and concludes that it is the result of the invagination of the outer endodermis through the leaf gap; or, to put it in another way, it is the persistence of the inner endodermis of the leaf traces of which the hollow stele is made up. The bundle at this stage most nearly resembles that of *Botrychium lunaria*, differing from that of *B. virginianum* in the absence of a true
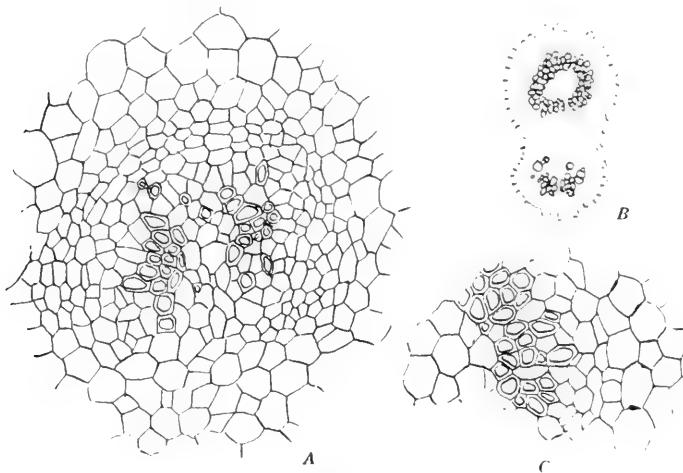


Fig. 52.

A. Section of the young central stele of *Helminthostachys*, showing the two xylems. × 200.
B. Stele from lower part of an older sporophyte, showing junction of a leaf trace with central stele. × 50.
C. Part of central stele, more highly magnified.

cambium, the outer wood cells being directly in contact with the inner cells of the phloem. Occasionally, however, there may be seen on the outer edge of the xylem ring a few imperfectly developed tracheids which probably represent a very rudimentary development of secondary wood, but there is no other sign of the definite cambium zone which is so conspicuous in the stele of *Botrychium virginianum*.

From this study of the development of the leaf traces, following them from the stem apex downward, it appears that the cylindrical stele in *Helminthostachys* arises in precisely the same way as that of *Botrychium*, viz. by the union of the leaf traces. The appearance of procambium upon the ventral side of the stele, which in longitudinal section appears to be derived directly from the stem apex, can thus be explained by the ventral extension of the broad leaf traces which meet on the lower side of the stem as well as above, and the cylindrical stele is thus developed.

It is not impossible that the root traces may also contribute to some extent to the development of this ventral portion of the stele. The xylem masses belonging to the two leaf traces remain quite distinct for a long time, the one belonging to the older trace being better developed than that from the younger one (fig. 52, *A*). The metaxylem consists of scattered tracheids arranged regularly in an arc outside the protoxylem of the older bundle, but there are a few scattered smaller ones lying in the tissue separating the two primary xylems, so that, as we have seen, the bundle may perhaps be described as mesarch, and not endarch as it is in *Ophioglossum* and *Botrychium*.

The two xylems of the separate bundles are finally connected by intermediate tracheary tissue, so that a nearly complete ring of wood is formed; but the xylem of the older leaf trace is still clearly recognizable on account of its greater thickness. In the section figured (fig. 52, *C*) there is no tracheary tissue yet formed inside the protoxylem and there is a well-marked pith occupying the center of the stele. The endodermis is now well developed and inside it are about two rows of pericycle cells. The protophloem is less conspicuous than in the younger stele and is best developed in the outer portion of the older leaf trace. The rest of the phloem is made up of
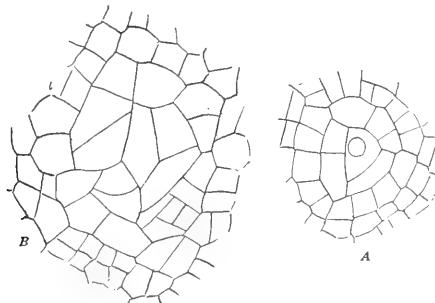


FIG. 53.

A. Transverse section of apex of first root of *Helminthostachys*, showing a single tetrahedral apical cell. ×200.
B. Apex of a later tetrarch root, with apparently no single initial cell.

thin-walled cells, the larger of which are presumably young sieve tubes. The section is taken from a much older part of the stem and shows that the bundle has attained practically the same condition as in the adult stem, except for the absence of the inner endodermis. The central pith is surrounded by an unbroken ring of wood, averaging about three cells in width. The outer part of the bundle is essentially the same as in the one that has been described.

The canal described by Gwynne-Vaughn for the older sporophyte is plainly evident here and extends backward from the sheath cavity, appearing in cross-section as a narrow cleft between the inner side of the leaf stalk and the internode (fig. 49, *D*, *y*). It is evidently not a cavity in the cortex, but merely an invagination of the epidermis, which is continuous with that of the inner surface of the stipular sheath.

A peculiar feature of the ground tissue in the young stem and petiole in *Helminthostachys* is the presence of cells containing roundish bodies which stain very strongly with Bismarck brown, and these cells closely resemble the tannin cells of the Marattiaceæ, with which they are probably homologous (fig. 49, *C*). Lang figures these cells (Lang 1, fig. 65), but makes no mention of them in his text.

The trace for the second leaf, the first functional one, is usually provided with a single undivided bundle passing through the petiole, but sometimes this divides into two, as it does in the cotyledon of *Botrychium virginianum*. Where a single bundle occurs in the petiole it occupies a nearly median position and is concentric in structure, although the phloem is somewhat less developed upon the adaxial side. The xylem consists of a large mass of tracheids surrounded by the phloem, which is reduced to about two rows of cells upon the adaxial side. Whether the largest cells in this portion were sieve tubes was not determined. Where two bundles are present they are somewhat smaller and are more nearly circular in outline than the single bundle, which they resemble in structure. In the ground tissue are large intercellular spaces which disappear at the base of the leaf, where the ground tissue in cross-section appears almost solid. This development of lacunæ in the first foliage leaf recalls the similar ones in the cotyledons of *Botrychium* and *Ophioglossum*.

In the older part of the rhizome there is a slight development of periderm on the dorsal side, which Farmer found to be the case also in the older rhizome. This periderm is restricted to the dorsal region and is obviously associated with the leaf bases, as it is in *Botrychium* and probably also in *Ophioglossum*. It presumably
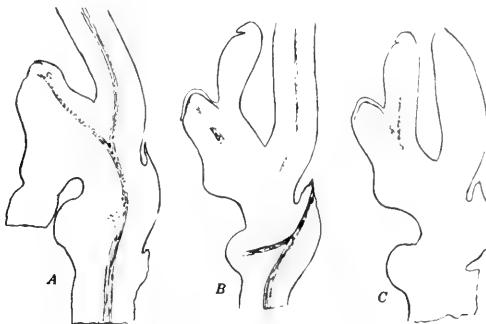


FIG. 54.

Three sections of an older sporophyte of *Helminthostachys*. x8. The stipular sheath is absent from the third youngest leaf.

acts as an absciss layer, such as Jeffrey demonstrated in *Botrychium*, and this not only causes the separation of the old leaves but perhaps also acts as a protective layer to the leaf scars. The absence of periderm from the ventral side is no doubt connected with the strictly dorsal position of the leaves.

The later roots, like the first one, grow from a single tetrahedral apical cell, very much like that of *Ophioglossum* and *Botrychium*. The root cap is not very prominent and is usually somewhat pointed. It apparently owes its origin entirely to the activity of the outer segments of the apical cell. The primary segments of the root cap undergo periclinal divisions, but, as in *Botrychium*, the stratifications of the older layers is much less marked than it is in the ordinary ferns. Each of the lateral segments of the apical cell divides by a somewhat radially placed anticlinal, so that a transverse section of the three youngest segments shows six cells having a nearly radial arrangement. Periclinal walls may arise and the inner cells of the segments give rise to the central cylinder of the root, while from the outer ones is developed the cortex. As in the other Ophioglossaceæ, no root hairs are formed.

The primary root, as Lang pointed out, is usually triarch, but occasionally diarch roots occur, as they usually do in *Botrychium* and in *Ophioglossum pendulum*. In this case, however, one of the xylems was rather larger than the other. The later roots are tetrarch and in the older sporophyte, as Farmer showed, the roots generally have six xylem masses, or occasionally seven. This type of root is most like that of the Marattiaceæ. The endodermis is pretty well developed, but not so conspicuous as it is in *Botrychium*.

In the cortical region of the first root there is a zone of cells in which occurs an endophytic mycorrhiza, such as is common in the roots of other Ophioglossaceæ, and probably the same as the endophyte which is found in the tissue of the prothallium. Whether the infection of the primary root is direct from the prothallium or whether there is a new infection from the soil was not determined. Lang states that the mycorrhiza is only developed in the first two or three roots, the fourth root and those formed later not having the endophyte. In these later roots the cortical cells are densely filled with starch.

The rhizome continues to grow upright for a good while and it was not determined at just what time it assumes the prostrate position which it has in the adult form. At first there is usually one root formed for each leaf, but in the older plants this regularity is lost, and Farmer states that there may be three or four roots developed for a single leaf. On the other hand, the number of roots may be less than that of the leaves, especially in the younger plants.

A curious abnormal form was seen in a young sporophyte, where for some reason several of the earliest leaves had remained in the rudimentary condition of the cotyledon. Five of these rudimentary leaves could be seen formed in succession. The sheaths were fully developed and there was a long internode between each pair of leaves. Three of these had developed roots, but the others had failed to do so. This rhizome was nearly 3 centimeters in length, but it had hardly increased at all in thickness. A single ternate leaf had expanded at the summit, but whether this was the first functional leaf that the plant had developed could not be determined, as the rhizome was broken off below and there may have been one or more functional leaves developed below the first of the rudimentary ones.

This repetition of vestigial leaves recalls the condition of things in *Ophioglossum vulgatum* and *Botrychium lunaria*. For a good while the new leaves are of the same ternate form as that of the first foliage leaf, but sooner or later, probably depending on the vigor of the plant, the ternate form is gradually replaced by five foliate leaves, the later divisions being the result of an unequal dichotomy of the lateral leaf segments, similar to that by which the second lateral segments of the primary leaf are separated from the terminal leaflet. In these five foliate leaves the characteristic "pecopterid" venation of the adult sporophyte is fully attained (fig. 45, *B*). Each lateral vein forks twice, the ultimate veinlets extending to the margin of the leaflet. A section of the petiole of one of these leaves shows that it contains four vascular bundles, arranged in pairs. The dorsal bundle of each pair is decidedly larger than the ventral one. The base of the petiole is almost perfectly cylindrical, but further up it becomes winged, so that a groove appears on its inner face extending for some distance below the junction of the lamina and the petiole. There is a very slightly developed hypodermal tissue composed of two or three layers of cells, the walls of which are colorless and considerably thickened, some of them showing thickened corners like the collenchyma found in the leaves of the Marattiaceæ.

### COMPARISON OF THE YOUNG SPOROPHYTES OF THE OPHIOGLOSSACEÆ.

If, as we believe, the type of sporophyte found in *Ophioglossum moluccanum* is really primitive, we may assume that the sporophyte at first had a single axial stele, collateral in structure and essentially the same in leaf and root. This primitive sporophyte had no stem at all, but consisted simply of leaf and root. From the primitive vascular skeleton, composed of a single unbranched strand, we can derive the different types characteristic of the older sporophyte in the three genera. As to the first origin of the stem apex, we can only conjecture; whether it originally arose, as it does in *Ophioglossum*, as an endogenous structure repeating, as it were, the origin of the primary root, we have no means of knowing, but this seems to be the most probable explanation of the origin of the stem apex in the primitive sporophyte from which are descended the different types of the Ophioglossaceæ. After the establishment of the stem apex the secondary leaves contributed their quota to the developing skeleton of the sporophyte. In *Ophioglossum* these leaf traces remain largely free and anastomose only to a limited extent, thus giving rise to the open tubular dictyostele with very large meshes. The structure of the individual strands of the dictyostele is essentially the same as that of the free leaf traces.

In *Helminthostachys* the early leaf traces fuse completely and there is formed a solid stele in the younger internodes with a central xylem core, composed of the united xylems of the two leaf traces. These leaf traces are approximately collateral in structure, although it may be that they have a small amount of phloem upon their inner face. After entering the petiole of the young leaf, however, these assume a distinctly concentric form. As the leaves increase in size their traces become broader and in section appear more or less crescentic, so that when the leaf traces come together there is left between them a certain amount of the ground tissue which after they have united appears as a pith lying inside of the tubular stele. This pith, however, it must be remembered, is not part of the stele proper, but is merely an included portion of the ground tissue. With the complete fusion of these two broad leaf traces the tubular form is established and the wood appears in section as a continuous ring. In *Botrychium*, especially in the large forms like *B. virginianum*, the tubular condition which is secondary in *Helminthostachys* is established at once; this is probably to be explained by the fact that the vascular bundles of the first leaves are much better developed, there being two strands in the cotyledon and in the second leaf, and the leaf traces belonging to these are correspondingly broad and on fusing include at once a certain amount of the ground tissue, so that the stele appears tubular from the beginning. *Botrychium virginianum* undoubtedly represents the most specialized type of the Ophioglossaceæ, and the development of the cambium with a permanent secondary thickening of the wood is an evidence of a higher degree of specialization in the vascular system than is found in any other living Pteridophyte. While a very slight indication of this secondary thickening has been found in *Ophioglossum*, and I have also noted some slight traces of it in *Helminthostachys*, there is never developed in these the genuine cambium ring, such as we find in *Botrychium virginianum*. In the development of the spiral protoxylem elements *Helminthostachys* differs from the other Ophioglossaceæ and suggests the true ferns. In the early development of its vascular system there rare strong suggestions of some of the Marattiaceæ, especially *Kaulfussia* and *Danæa*. The development of concentric bundles in the petiole in *Helminthostachys* and *Botrychium* also suggests the Marattiaceæ.

Assuming that the collateral bundle, which is typical of the stem in all of the Ophioglossaceæ and occurs also throughout in *Ophioglossum*, is primary, the con-

centric bundles as they occur in the petiole of *Helminthostachys* and *Botrychium* must be considered as secondary. The monarch root of *Ophioglossum moluccanum* and the other members of the section *Euophioglossum* is to be considered as a relic of the primary condition where the single axial stele, as in the young sporophyte of *O. moluccanum*, had the single strand of practically uniform structure, extending through the leaf and root. The development of diarch roots, such as those of *O. pendulum* and *Botrychium*, is probably also secondary and perhaps associated with the early development of the second leaf in these forms. The diarch root appears again in the young sporophyte of the Marattiaceæ and is permanent in most of the leptosporangiate ferns. The roots of the larger species of *Botrychium* and especially those of *Helminthostachys*, with their increased number of xylems, are undoubtedly secondary developments, perhaps associated with the large size of the roots; and we again find this same type developing in the Marattiaceæ. In regard to this point, *Helminthostachys* is the most aberrant of the Ophioglossaceæ and approaches nearest to the Marattiaceæ.

The leaf in the smaller species of *Ophioglossum* is probably a very primitive structure and the closed stipular sheath—which is not exclusively foliar in origin, but at first owes part of its tissue to that of the cortex of the root, from which the sporophyte arises—is probably an older structure than the strictly foliar, stipular sheaths in *Botrychium* and *Helminthostachys*. The simpler types of *Botrychium*, like *B. simplex* and *B. lunaria*, show a transitional condition between the closed sheath of *Ophioglossum* and the open sheath of *B. virginianum*, which may really be spoken of as composed of two stipules, in this respect recalling the Marattiaceæ. *Helminthostachys*, in the development of the stipular sheath, agrees exactly with the simpler types of *Botrychium*. In these forms the sheath is hood-shaped, opening by a transverse slit in front and below, and the upper portion of the sheath is broken through when the inclosed leaf emerges, so that the two apparent stipules in *Helminthostachys* are really secondary, caused by a tearing of this hood-shaped sheath, and are not proper stipules as they are in *Botrychium virginianum*.

The simpler and probably more primitive species of *Botrychium*, like *B. simplex* and *B. lunaria*, are obviously intermediate between *Ophioglossum* and the larger and more specialized species of *Botrychium*. This is shown in the form and venation of the leaves, as well as in the character of their tissues. Instead of the pinnate venation found in the leaves of *B. virginianum*, these more primitive species show no midrib in the leaf, but the veins all radiate from the veins of the leaflet, dividing dichotomously, so that they are arranged in a fan-like fashion. Were the ends of the veins connected there would result a reticulate venation, exactly like that of *Ophioglossum*. These leaves, moreover, are fleshy in consistence and have stomata upon both sides, while in *Botrychium virginianum* and in *Helminthostachys* the stomata are restricted to the lower surface of the leaf. In all of these particulars *Ophioglossum* and the simpler species of *Botrychium* are evidently more primitive than the other genera.

In *Botrychium virginianum*, as we have seen, the cotyledon is extraordinarily developed, more so than that of any other Pteridophyte. The contrast between this highly developed cotyledon and the very rudimentary one in *Botrychium lunaria*, where the young sporophyte passes several years under ground before the first green leaf emerges, is very striking. It is, however, to be assumed that the rudimentary condition of the leaves in *Ophioglossum vulgatum* and *Botrychium lunaria* is a secondary condition, connected with their long life under ground.

*Helminthostachys*, on the whole, approaches more nearly to *B. lunaria* in its early stages of development than it does to *B. virginianum*. This is shown in the

rudimentary condition of the cotyledon and in the venation of the first foliage leaf, which, although it has a ternate lamina, is quite rudimentary. We have already referred, however, to the rudimentary leaves of *B. lunaria*, which also sometimes develop a very small ternate lamina. The venation of the first leaf in *Helminthostachys* also approaches the cyclopterid type found in the leaves of *B. lunaria*. The structure of the stele in the young stem also, after attaining the tubular form, is more like that of *B. lunaria* than like that of *B. virginianum*.

On comparing the early stages of development of the Ophioglossaceæ and Marattiaceæ we shall find that they have a good many points in common, which will be discussed more at length after we have described the structures of the latter. *Ophioglossum*, as to the formation of the cotyledon, is very much like *Kaulfussia*, while *Helminthostachys* is more suggestive of *Danæa*. The stipular structures of the two families are unquestionably homologous and the entire conical stipular sheath, found in the lower members of the Ophioglossaceæ, is probably an older type than the free stipules found in *Botrychium virginianum* and the Marattiaceæ.

In both the Ophioglossaceæ and Marattiaceæ the young sporophyte at first is made up almost entirely of leaf and root, and the whole vascular system is composed of the leaf traces with no proper cauline vascular tissues, so that one might almost say that the stem is made up entirely of the bases of the leaves, the dominance of the leaf being the most noteworthy feature in the morphology of these plants. On the whole, probably *Helminthostachys* most nearly resembles the Marattiaceæ. This is true both of the character of the venation of the leaves, the structure of the root bundles, and the development of tannin cells in the young sporophyte. These tannin cells seem to be quite absent from the tissues of both *Ophioglossum* and *Botrychium*.

# IV. THE ADULT SPOROPHYTE.

The essential characteristics of the sporophyte are established while it is still quite small and the subsequent differences are mainly an increase in the size of the parts and finally the development of the spore-bearing structures which constitute the peculiar spike or sporangiophore so characteristic of these plants. As the structure of the tissues has been repeatedly studied and described, no attempt will be made here to take up a detailed study of these.

The Ophioglossaceæ in general, except for the rather elaborate vascular bundles of the stem, especially in *Botrychium*, are marked by great simplicity in the structure of the tissues. The surface is usually smooth except in the younger plants, where there may be a development of scales and hairs, presumably for the protection of the young tissues of the stem apex. The hard hypodermal tissues and bands of sclerenchyma, so common in the leaves and stems of many ferns and developed to a less degree in the Marattiaceæ, are practically entirely absent from the Ophioglossaceæ.

Except for the vascular system of the stem the bundles are usually less developed than is common in the more highly differentiated ferns, this being especially the case in *Ophioglossum*, where the very delicate bundles forming the veins of the leaf run through the spongy green tissue of the leaf without causing any projecting veins at the surface. This condition is true also in the simpler types of *Botrychium* and in the young leaves of *Helminthostachys*. The great bulk of the ground tissue in leaf, root, and stem is parenchyma. The development of periderm in the outer tissues of the stem is probably always associated with the leaf bases and serves, as we have seen, both to separate the dead leaf bases and to protect the scar thus left after the leaf has fallen away.

The roots are characterized by the complete absence of root hairs. The outermost layer of cells often has the walls much thickened and they may show the reaction of cork. The bulk of the cortex of the root, however, is composed of unmodified parenchyma. The vascular cylinder in the root remains monarch in the section *Euophioglossum*, but is diarch in the smaller roots of *Ophioderma* and in the smaller species of *Botrychium*, and ranges to hexarch in the larger roots of *Helminthostachys*. The roots, especially in the larger species, are thick and fleshy and as a rule branch sparingly and somewhat irregularly. In the section *Euophioglossum* no lateral roots are formed and branching is rare. When it does take place it is the result of a true dichotomy of the apex. The root, as in the youngest stages in the plant, grows almost always from a single tetrahedral apical cell, which is much alike in all the genera.

The most characteristic feature of the Ophioglossaceæ is the peculiar sporangial spike referred to. There is a certain correlation in the degree of development of this spike and the sterile leaf segment with which it is associated. The fertile leaves may arise very early in the history of the sporophyte. Bruchmann states that the first leaf to appear above ground in *Botrychium lunaria* is already a fertile one and in *Ophioglossum vulgatum* the second green leaf to be developed usually bears spores. This early development of the fertile leaf is probably an indication of the primitive nature of these plants, as we must assume that the ancestral form must have at once developed a sporangial structure on the first leaf, or what corresponded to that in the embryo. In *Botrychium lunaria* Bruchmann figures young spore-bearing plants which are still connected with the prothallium, and Jeffrey states that in *B. virginianum* he once found a fruiting plant with which the prothallium was still connected.

## THE SPOROPHYTE OF OPHIOGLOSSUM.

Of the three existing genera of the Ophioglossaceæ, the genus *Ophioglossum* is much the largest and most widespread. The number of species, however, is very imperfectly understood, as there is great confusion in the nomenclature, owing to the inadequate study of the tropical species, of which the number is undoubtedly much greater than has generally been supposed. The smaller terrestrial species look very much alike and it is evident that collectors have often failed to discriminate among them. My attention was especially called to this while I was collecting material of *Ophioglossum* in Java, where there is evidently a considerable number of species. Raciborski (Raciborski I), who has described the Pteridophytes of this region, mentions only a single species as occurring in the neighborhood of Buitenzorg, where most of my collecting was done. But in that immediate neighborhood I collected at least three very distinct species, and two were collected at Tjibodas, some 4,000 feet above Buitenzorg. Raciborski, however, mentions only a single species as occurring at Tjibodas. There is no doubt that a critical study of these plants from other regions where they abound will greatly increase the number of species to be recorded.

The great majority of the species of *Ophioglossum* belong to the subgenus *Euophioglossum*, which should probably be separated completely from the very different forms which comprise the other subgenera. The species of *Euophioglossum* are all small or moderate-sized plants with undivided leaves which are generally lanceolate or broadly oval in outline and have the sporangial spike borne on a very long stalk attached to the sterile part of the leaf near the base of the lamina. These plants are always terrestrial, the leaves growing from an upright rhizome, which is sometimes a good deal enlarged, sometimes more slender in form. From the rhizome there extend numerous roots which are sometimes developed one for each leaf, but this is by no means always the case. The sporophyte of *Euophioglossum* seldom exceeds a height of 30 to 40 centimeters and some of them are very much smaller (fig. 55, also plate 3).



Fig. 55.

Two specimens of *Ophioglossum moluccanum* (Schlecht), slightly reduced. The larger one is the typical *O. moluccanum*; the smaller one is probably a second species.

Except for their larger size the later leaves do not differ essentially from the primary leaf of the young sporophyte. The venation is always reticulate, usually without any definite midrib, although sometimes there is a central vein which is slightly stronger than the others. In many species, e. g., *O. moluccanum*, *O. vulgatum*, small branches ending blindly are found within the large meshes. In other species, e. g., *O. lusitanicum*, there are no free veins within the areoles.

In many of the species but one leaf is formed each year, but in others, especially many of the tropical species, there may be as many as four or five formed in a

single season. In *O. vulgatum* the development of the leaf is very slow, the leaf remaining three years inclosed in the bud and emerging in the fourth season. So far as I know, no study has been made of the development of the leaves in those species of the temperate climates where more than one leaf is developed in the season, and it remains to be seen whether the leaves which unfold in the same season are of the same age or not. In the rapidly growing tropical species it is exceedingly unlikely that more than a few months are necessary for the complete development of the leaf, which unfolds as soon as it is mature, but no data are available on this subject.

While the spike in *Euophioglossum* is usually inserted at the base of the sterile lamina it may be attached much further down. This is especially noticeable in the small species *O. bergianum*, from South America (see Bower **9**, page 435). In *O. bergianum* the leaves do not show a clear separation into lamina and petiole and the spike is inserted close to the base of the linear leaf, so that it appears to be an entirely distinct structure.

In nearly all of the species of *Euophioglossum* the later roots, like the primary ones, are monarch, but in *O. bergianum* they are diarch. In most cases at least, as Holle (Holle **1**) showed, only one root is formed for each leaf. This seems to be the case in *O. moluccanum*, which was investigated with some care.

*Ophioglossum pendulum* (plate 4, B) is the best-known representative of *Ophioderma*, the second section of the genus. This differs a good deal in its general characters from the small terrestrial species of *Euophioglossum*. It is not uncommon throughout the eastern tropics, where it grows as an epiphyte upon the trunks and branches of various trees. The rough stems of certain tree ferns and some palm-like species of *Phœnix* form a favorite habitat for this fern in certain regions and, as we have pointed out in the study of the gametophyte, it is frequently found rooting in the masses of humus between the old leaf bases of some epiphytic ferns like *Asplenium nidus*. The stem, instead of being upright, is markedly dorsiventral, but is short, with the leaves crowded together near the end and all growing upon the dorsal side of the rhizome, as they do in *Helminthostachys* (fig. 63). The leaves are very much elongated and the ribbon-like lamina merges very gradually into the extremely stout petiole. These leaves in large specimens may reach a length of 1.5 meters, or even more, and the long, strap-shaped leaves hanging down from the boughs of the trees present a very characteristic appearance. In larger specimens it is not uncommon to find the lamina dividing dichotomously (see plate 4, B) and it is said that sometimes this dichotomy may extend to the petiole, in which case each segment of the leaf bears a separate spike.

The venation of the leaf is reticulate, the veins inclosing long, narrow meshes with no free veins, and the venation thus resembles the type represented in *Euophioglossum* by *Ophioglossum lusitanicum*. The larger plants always have several leaves which are expanded at the same time and growth goes on uninterruptedly. The leaves are crowded together and there is no evident internode between them. but it is clearly seen that they are arranged in two rows on the flanks of the short rhizome (fig. 63, *A*). The remains of the stipular sheaths surround the bases of the leaves and these sometimes present the appearance of two small stipule-like organs. As in *Euophioglossum*, adventitious buds are frequently formed upon the roots. As a rule they are not terminal, but lateral structures and probably do not differ essentially in their development from those of *O. moluccanum*.

The rhizome is more or less buried in the humus and the roots ramify through this. Even in the very young plant the roots are branched and this branching becomes very marked in the larger sporophyte. There seems to be some difference

in the number of roots, but in most cases two were found connected with each leaf, instead of one as I stated in my earlier description of the plant (Campbell 4). The roots are very stout, sometimes attaining a diameter of over 3 millimeters. The smaller roots are diarch, but in the large roots there may be three, four, or even five xylems.

*Ophioglossum pendulum* is much the largest member of the Ophioglossaceæ and the sporangial spike as well as the individual sporangia far exceeds in size those of any other species (plate 4, B, 3–6). The spike is attached by a short peduncle and hangs down from the pendent lamina. In large specimens the spike may reach a length of 20 centimeters or more with a breadth of about a centimeter. Above the insertion of the spike the leaf is thin, but the slender peduncle is continued downward into a thick, flattened midrib which merges gradually into the petiole, so that the spike has very much the appearance of being a terminal structure with the sterile lamina adherent to it.

The young leaves in *O. pendulum* emerge while they are still very young and the sporangial spike is in an extremely rudimentary condition The general development of the leaf is therefore very easy to follow in this species, as these young leaves are entirely free.

The second species of the section *Ophioderma* is the rare *O. intermedium* (fig. 69; plate 4, A). This for a long time was known only from one locality in Borneo, but has lately been collected at other points in the Malayan region. The specimens figured were collected by the writer near Buitenzorg in Java. In the account of the Ophioglossaceæ given in Engler and Prantl's "Natürliche Pflanzenfamilien" (Bitter 2) it is suggested that *O. intermedium* is only a terrestrial form of *O. pendulum;* but there is no doubt at all that it is a very distinct species. The plants described here were growing in masses of humus at the base of old clumps of bamboo. The stem is usually very short, forming a small tuberous body, from which, in most cases, only a single leaf was growing, although two were sometimes found. A careful examination of this short rhizome showed that, like *Ophioglossum pendulum*, it is dorsiventral. The roots were short and in all cases observed were without branches. In one of the specimens a bud very similar indeed to the corresponding buds in *O. pendulum* was found growing from one of the roots. *Ophioglossum intermedium* differs from *O. pendulum* in being rigidly upright. The peduncle is longer and the lamina of the leaf much smaller and more clearly differentiated from the petiole. As in *O. pendulum*, however, the petiole is prolonged into the peduncle of the spike with the same midrib-like thickening, caused by the coherence of the basal part of the peduncle with the lamina.

This plant is exceedingly variable. In the larger specimens, except for the much shorter lamina, the plant a good deal resembles small specimens of *O. pendulum*. In others, however, the lamina is almost completely suppressed and this condition closely approaches the third member of this section, *Ophioglossum simplex* (fig. 71). This latter species is at present known only from one locality in Sumatra, and is distinguished from all the other species of *Ophioglossum* by the practically complete suppression of the sterile portion of the leaf (see Bower 8). The form of the rhizome and the habit of the plant in *O. simplex* most nearly resemble *O. intermedium*, to which it is probably not very distantly related. Whether or not we regard the absence of the lamina of the leaf as a case of reduction, the fertile leaf in *O. simplex* certainly very closely resembles what one would assume to have been the primitive condition of the leaf in the ancestors of the Ophioglossaceæ.

The third section, *Cheiroglossa*, contains a single very peculiar species, *O. palmatum* (plate 5). This occurs throughout tropical America, but does not seem to be a common plant. It has been reported from Florida, where, however, it is

very rare. It has been collected in various parts of South America and in the West Indies, and apparently the same species is known also from the Island of Bourbon and from the Seychelles in the Indian Ocean. The specimens from which the figures and descriptions given here were made were collected in the summer of 1908 in the Blue Mountains of Jamaica, and were found in one place only, growing upon a rotten tree stump.

The rhizome in *Ophioglossum palmatum* is an almost globular tuber, which is radially constructed and in this respect resembles that of *Euophioglossum* more than that of *Ophioderma*. The leaf bases are covered with long brown hairs, which give a characteristic appearance to the rhizome. The roots are numerous and more slender than those of *Ophioglossum pendulum*. They are sparingly branched and the branching may have the appearance of a true dichotomy, but the material available did not allow of a critical investigation of this point. However, since in *Euophioglossum* the branching of the roots is actually dichotomous, it is not impossible that the same may be true in *Cheiroglossa*. The smaller roots, as in *O. pendulum*, are diarch and, as in all of the other forms, a mycorrhiza is developed in the cortical region.

In sections of one of the largest roots available the bundle was triarch. This root was rather young and the tracheary tissue was only partially lignified. The mycorrhiza was also less evident than in the smaller roots taken from a younger plant. Two young plants were found which probably had arisen from buds upon the roots of the older sporophyte. One of these is shown in figure 70, *A*. It had developed a single nearly lanceolate leaf very much like the early leaves of *O. pendulum*. A single root had developed near the base of the leaf and this was joined to the small globular rhizome. A second root could be seen above this, but whether this second root belonged to the leaf or was part of the root upon which the bud had arisen could not be determined.

The older leaves may reach a length of 60 centimeters or more (plate 5). They are more or less deeply palmately cleft into narrow segments, which give the leaf a curious superficial resemblance to certain kelps, such as *Laminaria digitata*. The base of the fan-shaped lamina is abruptly narrowed into a nearly cylindrical petiole, about equal in length to the lamina itself. The venation is rather of the type of *O. vulgatum*, having numbers of free veins in addition to the elongated closed meshes.

In some of the smaller leaves (plate 5, 3) there is present a single small median spike, which, like that of *O. pendulum*, has a short peduncle, but it is inserted below the lamina, in this respect again being more like *Euophioglossum*. In the larger leaves there are several sporangial spikes arranged in a series on either side of the petiole, usually below the insertion of the lamina, but sometimes some of them are inserted above the junction of the lamina and the petiole. Bower (Bower **9**, page 436) has figured a series of specimens of this species showing the great variation in the number and arrangement of the spikes. He emphasizes the fact that although these spikes are apparently marginal, in reality they always arise from the adaxial side of the leaf. He shows that in this species also there may be a branching of the individual spikes similar to that which occurs in *Ophioglossum pendulum* and less frequently in certain species of *Euophioglossum*.

### THE ANATOMY OF EUOPHIOGLOSSUM

The stem apex of the adult plant has been repeatedly studied in the widespread *Ophioglossum vulgatum*, but there is little information in regard to this point in other species. From a somewhat careful examination of *O. moluccanum* it is evident that this species agrees closely with *O. vulgatum*, and a study by one of my students,

Mr. C. S. Morris, of this species and also of a broad-leaved form from Ceylon,
probably *O. reticulatum*, showed that the same characters prevail in the species he
examined as in *O. vulgatum*. It therefore seems likely that all species of *Euophio-
glossum* agree in the main in the structure of the stem apex and the development
of their tissues.

We have seen that in the young sporophyte the stem apex is completely inclosed
in a small cavity formed by the hood-like stipular sheath of the youngest leaf. This
condition, once established, is permanent (fig. 56) and there is no essential difference
in the appearance of the stem apex between a young plant in which two or three



Fig. 56.

Longitudinal sections of an older sporophyte of *Ophioglossum moluccanum*, showing arrangement of
vascular bundles; *x*, stem apex; *sp*, young sporophylls. D shows apical region more enlarged.

leaves have been developed and the full-grown sporophyte. The stem apex is of
limited extent, crowded in between the bases of the young leaves, and in *O. moluc-
canum* the single apical cell is of the same form as that which was found in the early
condition of the young bud; i. e., in longitudinal section it is four-sided, with a
broad base and a narrower outer face. According to Holle (Holle 1), the apical
cell in *O. vulgatum* is pointed below; but Rostowzew (Rostowzew 2) in his figures
shows the same form as the apical cell in *O. moluccanum*. It is not at all impossible,
however, that both forms may occur, as in cross-sections the apical cell in *O. mol-
uccanum* may be either three-sided or four-sided (figs. 39 and 56, *D*). The apical

cell in *O. moluccanum* may therefore be described as a three-sided or four-sided prism or a truncate pyramid.

As in the young plant, the stipular sheath of each new leaf is formed mainly from the basal tissue of the leaf itself, but includes also tissue from the margin of the stem apex. The sheath is open above by a narrow pore through which it communicates with the space between it and the next stipular sheath. In *O. moluccanum*, as in *O. vulgatum*, three leaves of different ages can generally be seen inclosed in the bud and sometimes the first indication of the fourth young leaf can
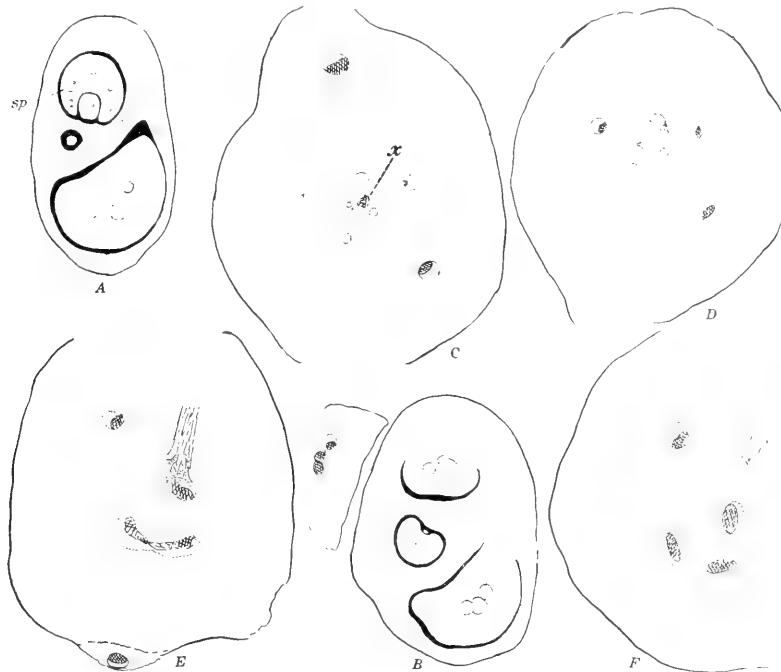


Fig. 57.

Six of a series of cross-sections from a sporophyte of *Ophioglossum moluccanum*. *sp*, young sporophyll; *x*, stem apex.

also be distinguished (fig. 56, *C, D*). A cross-section of the youngest sheath shows the pore as a minute opening, separated from the stem apex below by a very narrow space.

A careful study of the older stem gives no reason for assuming that the tissues of the very open reticulum forming the fibrovascular system of the rhizome is in any part due to additions from the apical tissue of the stem. Whether longitudinal or transverse sections are examined it is perfectly clear that the strands of this reticulum are made up entirely of leaf traces which can be readily followed into the young leaves or the tissue immediately below. The immediate apical region of the stem, as in the younger sporophyte, is unmodified parenchyma which adds only to the large central pith, if pith this can be called, as the separate bundles of which the vascular reticulum is composed run free for long distances through the ground tissue

of the stem, which is identical in appearance in the cortical and the central regions.
The bundle from each young leaf can be traced to a junction with a root stele and
from this point of junction it extends through the ground tissue of the stem, running
almost horizontally until it joins the trace from the next older leaf. In this way is
built up the open, large-meshed vascular cylinder. So far as could be determined
in *O. moluccanum* only one root was formed for each leaf. The tissues of the root
base are continued upward to connect with the young leaf and downward to join the
stele from an older one.

No endodermis can be detected in the bundles of the stem in *O. vulgatum* and
the same is true for *O. moluccanum*, but in *O. bergianum*, *O. capense*, and *O. ellip-
ticum*, according to Poirault (Poirault 2), both an inner and outer endodermis occur
in the older part of the rhizome; these however, disappear in the younger region
higher up.

A transverse section of the mature rhi-
zome (fig. 57) in *Ophioglossum moluccanum*
shows the widely separated sections of the
strands of the vascular cylinder as a circle
of small collateral bundles without any
endodermis, the mass of wood being in
immediate contact with the thin-walled
parenchyma of the ground tissue or sepa-
rated from it at most by a single row of
pericycle cells. In some of the smaller
species the leaf traces are relatively broader
and there is an approximation to the ring-
shaped section presented by the cylindrical
stele of *Botrychium* or *Helminthostachys*,
and sometimes the same appearance may be
approached in sections of the older rhizome
which happen to pass through a region
where there are numerous anastomoses of
the bundles forming the reticulum (fig. 57, *E*).

Except for the vascular bundles, the
tissue of the stem is made up almost ex-
clusively of simple parenchyma. The de-
velopment of periderm, which takes place
to a limited extent in the outer region, is
doubtless associated with the old leaf bases,
as it is in *Botrychium*.

The leaf structure of *Euophioglossum* is
exceedingly simple. The mesophyll is made
up of thin-walled green cells, practically uni-



Fig. 58.

Longitudinal section of a sporophyte of *Ophioglossum
moluccanum*, older than the one shown in fig. 56. × 20.

form throughout; and through this spongy mass of mesophyll the delicate veins pass
without forming any projections at the surface. Both sides of the leaf are provided
with a simple epidermis, stomata being developed on both sides of the leaf. In
those species in which the leaf lies more or less horizontally, as it does, for example,
in *O. reticulatum*, the stomata are less numerous upon the upper surface.

The arrangement of the bundles in the petiole has been already studied in the
commoner European species, *O. vulgatum* and *O. lusitanicum*. In all of the species
belonging to the section *Euophioglossum* there is given off from the vascular system
of the rhizome a single leaf trace, which divides at the base of the leaf into two

strands. Each of these strands may divide, or only one of them (fig. 60). In some of the smaller species there are only three bundles in the petiole, and in these forms there are no anastomoses of the bundles above the base. In the larger species these branches divide further and the number of bundles in the section is greater. Thus, each of the two ventral bundles may divide again in the upper region of the petiole so that a cross-section at this point would show five bundles, a large dorsal one and two pairs of smaller ventral ones. A similar condition of things is found in the
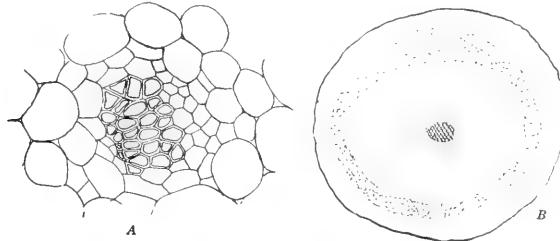


FIG. 59.

A. Vascular bundle from an adult rhizome of *Ophioglossum moluccanum*. ×150.
B. Section of an adult root showing monarch vascular cylinder. ×35. The shaded zone is occupied by the mycorrhiza.

small species, *O. californicum*. As sections are examined below the junction of the lamina and the petiole, it can be seen that further forking of the bundles has taken place preliminary to their entering the lamina itself. Some of the species have the tissues of the petiole quite compact, others show a greater or less development of lacunæ or air-spaces in the petiole.

### THE ROOT IN EUOPHIOGLOSSUM.

The early roots in *O. moluccanum* grow from a large tetrahedral apical cell showing a fairly regular segmentation, much like that of the typical ferns; but the root cap is not so well developed nor does it show as definite a stratification. In the
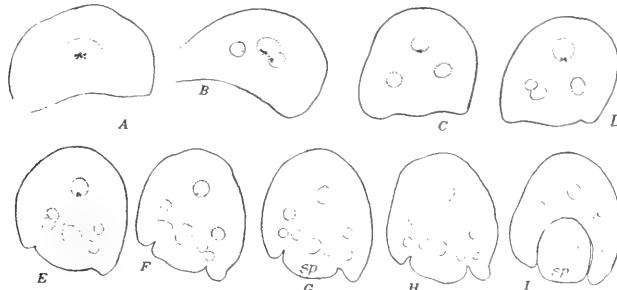


FIG. 60.

Nine of a series of sections of a young sporophyll of *Ophioglossum moluccanum*. *sp*, the sporangiophore.

early roots the whole of the root cap seems to be derived from the outer segments of the apical cell. In the later and larger roots there seems to be somewhat less regularity in the segmentation and no sections were found which showed the apical

cell and its segmentation as clearly as in the younger roots; however, only a small number of these later roots were sectioned and it may be that they were not all quite normal.

A section of an adult root is shown in fig. 59, *B*. The cortical region is made up of simple parenchyma and the mycorrhizal zone is very conspicuous. The endodermis is not very clearly defined, less so than in the earlier roots, and its limits are
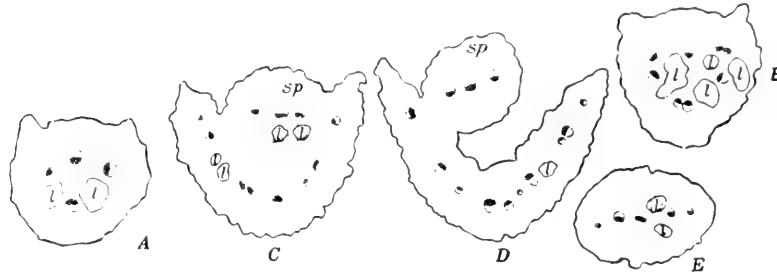


FIG. 61.

Five sections of a full-grown sporophyll of *Ophioglossum moluccanum*. E, section of peduncle of spike; *l*, lacunæ.

not easily determined. The cortical cells inside the mycorrhizal zone are densely filled with starch. More than half of the section of the bundle is occupied by the large solid mass of wood. This is separated from the endodermis by a single layer of pericycle cells. The single mass of phloem lying next the wood is not much more than half as large.

ANATOMY OF OPHIODERMA.

Owing to the dorsiventral position of the rhizome and to the more rapid development of the leaves, the section of the stem apex in *Ophioglossum pendulum* differs a good deal from a similar section of *Euophioglossum* (fig. 64). The base of the leaf is bent sharply upward, this being the case also in the very young leaf which is inclosed within the bud. The leaves break through the inclosing sheath in a very rudimentary condition, although of such size that a section through the base of the youngest visible leaf shows usually but a single younger leaf inside the sheath, instead of the three or four that are seen in a similar section of the bud in *Euophioglossum*. Within the sheath at the base of the youngest visible leaf there is found a cavity in which is the growing point of the stem and the youngest leaf. The apex of the stem forms a strongly inclined, nearly plane surface and, as in *Euophioglossum*, the growth is from a single apical cell which, so far as could be determined, has the form of a three-sided pyramid, whose



FIG. 62.

Apex of root of *Ophioglossum moluc-canum*, showing dichotomy. × 150.

lateral faces are more or less strongly convex and whose apex may be truncate. From the few cases observed it is not possible to say definitely whether basal segments are regularly cut off as well as the lateral ones. The segments cut off from the lateral faces are large and the divisions irregular.

Owing to the large size of the sporophyte there are practical difficulties connected with the study of the vascular system of the rhizome from a series of microtome sections. The numerous bundles in the leaf base do not unite into a single

leaf trace, but pass downward through the cortex separately, and even if it were possible to make a satisfactory series of the sections of the large rhizome the task of following the course of the very numerous individual bundles would be a difficult one. From free-hand sections of the large base of the leaf, however, one can see without difficulty the general plan of the vascular skeleton. The stout petiole is slightly contracted at its base, but a section presents very much the same appearance as one made higher up.

In specimens that have been preserved in alcohol so as to become decolored, the tissues of the thick leaf base are sufficiently transparent to show quite clearly the course of the bundles even without sectioning, by simply slitting the petiole through the middle. The bundles are seen to anastomose freely at the extreme base of the leaf, very much as they do in the lamina. If such a section is examined where the leaf base joins the rhizome (fig. 63, *D*) the circle of bundles can be seen to become
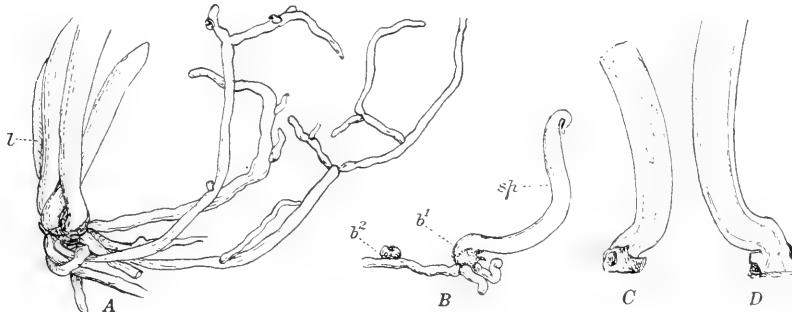


FIG. 63.—*Ophioglossum pendulum* L.

A. Stem showing dorsiventral form and branched roots. *l*, bases of leaves.
B. Buds attached to a root fragment. The older bud, *b*$^1$, has developed a young sporophyll, *sp*, and two secondary roots.
C. Base of a large leaf, showing arrangement of vascular bundles.
D. Section of same.

smaller as the bundles enter the cortex, but the bundles remain for the most part quite separate, although there may be an occasional anastomosing of these, as occurs in the petiole itself. This group of bundles composing the leaf trace—if such it can be called—forms a circle about the opening in the vascular cylinder of the stem where it joins the latter. A section of the vascular cylinder within the rhizome itself presents the appearance of a nearly complete ring, which is probably formed by fusing of the bundles derived from the leaf bases. The much greater development of woody tissue in the stem of *O. pendulum* as compared with that of *Euophioglossum* is doubtless due to the very much larger size of the leaves and the correspondingly greater number of vascular bundles contributed by them to the woody cylinder of the rhizome.

The very young leaf, such as is shown in fig. 63, *B*, has a thick, fleshy leaf base, terminating in a very small and pointed lamina strongly bent over as it is, e. g., in the young leaf of *Botrychium virginianum*. This differs from the species of *Euophioglossum*, in which the leaf is straight in the bud. Under the arching hood formed by this folded-over lamina is the young spike, almost equal in length at this time to the lamina itself. A somewhat older stage is shown in fig. 82, *B*. The leaf has now become somewhat flattened, but as yet there is no sharp distinction between the lamina and the petiole. In this early stage of development the interpretation of the spike as a terminal structure seems very plausible. An interesting

case is shown in fig. 82, *E*, where the lamina is almost entirely suppressed and the terminal character of the spike is very evident.

As the leaf develops, there is a very great increase in size in the lamina, which, as we have seen, may reach a great length. The anatomical structure of the leaf
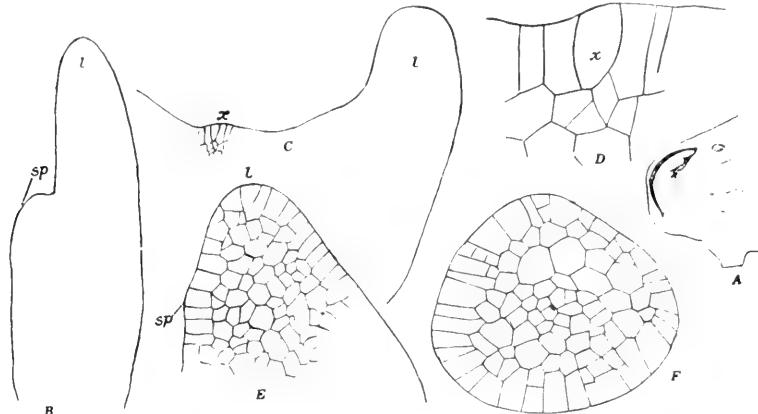


FIG. 64.—*Ophioglossum pendulum.*

A. Section of stem apex. ×3.
B. Young sporophyll. *sp*, sporangiophore. ×35.
C. Stem apex, *x*, and youngest leaf, *l*.
D. The stem apex, more enlarged.
E. Very young sporophyll. *sp*, sporangiophore. ×90.
F. Cross-section of young sporangiophore.

closely resembles that of *Euophioglossum*, but the stomata are much larger and around the stoma is a concentrically arranged series of cells, indicating that probably the formation of the mother cell of the stoma is preceded by a series of preliminary
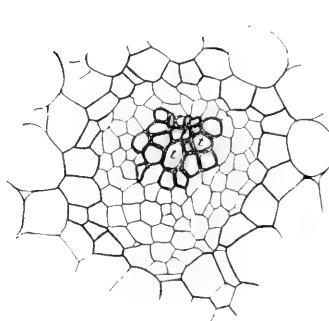


FIG. 65.—Vascular bundle from petiole of *Ophioglossum pendulum*.



FIG. 66.
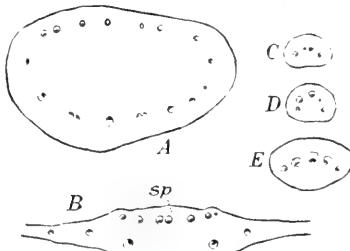
A. Section of petiole of a sporophyll of *O. pendulum*.
B. Section near base of lamina. *sp*, base of spike.
C–E. Sections of peduncle of spike. C and D are from the same leaf; E from a larger one.

divisions in the epidermal cell. The tissues in the petiole are very like those in the lamina of the leaf except that they are somewhat more compact. The walls of the ground tissue in this region are very deeply pitted.

The structure of the vascular bundle of the petiole (fig. 65), is much like that in *Euophioglossum*, except that there is a rather greater development of tissue upon the

inner side of the wood, so that there is a slight suggestion, at least, of a concentric structure of the bundle, such as is found in the petioles of *Botrychium*. The xylem is composed entirely of tracheids with reticulate markings. The rest of the bundle is made up of sieve tubes, mingled irregularly with smaller, thin-walled elements. It



could not be certainly determined whether any sieve tubes were developed on the inner side of the wood.

The number of bundles in the petiole, especially in large specimens, is much greater than in any of the species of *Euophioglossum*. In a specimen of medium size (fig. 66) eighteen bundles could be seen, of which probably seven or eight are destined to supply the spike. In the fertile leaves these form a complete circle, but in sterile leaves there are no bundles on the
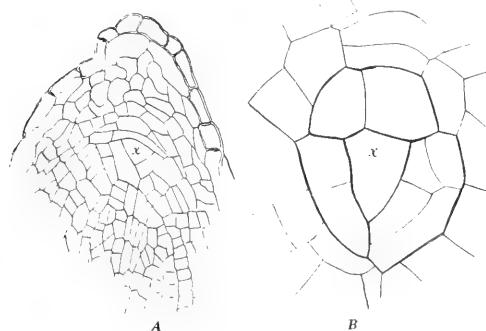
FIG. 67.

A, longitudinal; B, transverse section of the root apex of *Ophioglossum pendulum*

adaxial side of the section. In the free portion of the peduncle, which is relatively slender, the number of bundles is much less than in the broad basal portion. In the smaller specimen figured there were but three bundles in the middle part of the peduncle. The complete absence of the adaxial strands in the petiole of the sterile leaf, even at its base, is a strong confirmation of the view suggested by both the older leaf and the younger stages, that the peduncle is really an independent structure whose bundles are joined directly to the rhizome.
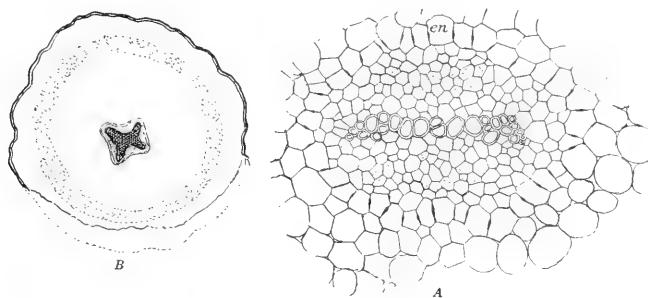


FIG. 68.

A. Diarch root-bundle of *Ophioglossum pendulum*.
B. Tetrarch root of the same. ×20. Dotted area is mycorrhizal zone.

*O. intermedium* (fig. 69) closely resembles *O. pendulum* in its anatomy, like the latter developing stomata upon both sides of the leaf, and these stomata are very large and show accessory cells similar to those found about the stomata of *O. pendulum*. Except for the difference in size, the arrangement of the vascular bundles in the peduncle and their structure is closely similar to that in *O. pendulum*.

7

The anatomy of *O. simplex*, so far as it has been investigated (see Bower 8) corresponds closely with that of the other species of the section *Ophioderma*.

The roots of *O. pendulum* also show the tetrahedral apical cell (fig. 67), but the divisions are much less regular than in *O. moluccanum* and probably the other species of the *Euophioglossum*. The segments divide slowly and increase a good deal in size before the first divisions take place, so that the youngest segments may equal the apical cell in size, and there is thus a certain resemblance to the apical meristem in the root of the Marattiaceæ. It is probable that, as in the Marattiaceæ, the lateral segments of the apical cell also contribute to the root cap, which is rather better developed than it is in *O. moluccanum*.

There is a good deal of difference in the structure of the root bundle (fig. 68). This is diarch in the primary root, as we have seen, and also in the smaller ones of older sporophytes; but in the larger roots, which may reach a diameter of over 3 millimeters, it is triarch; tetrarch bundles are also common, and in one very large root which I examined the bundle was pentarch,



Fig. 69.

Five specimens of *O. intermedium* Hk., slightly reduced.
C and D have a very much reduced lamina.

but one of the xylem rays was shorter than the others, the xylem forming a solid central mass having the form of an irregular five-pointed star.
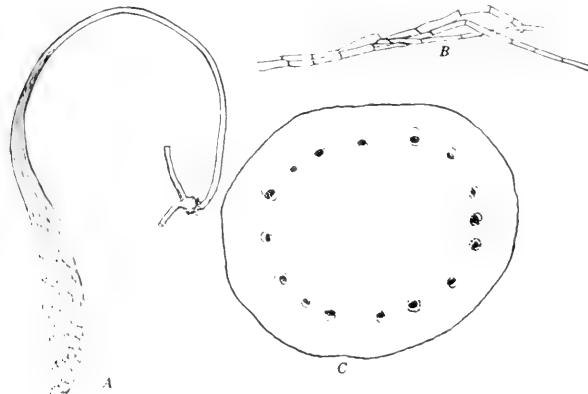


Fig. 70.

A. Small plant of *Ophioglossum palmatum* L., growing as a bud upon a root fragment
B. Hairs from base of leaf.  ×35.
C. Section of petiole of a large leaf.  ×7.5.

## ANATOMY OF CHEIROGLOSSA.

Material was lacking for a study of the anatomy of the rhizome in *O. palmatum* (fig. 70), but the structure of the petiole is very much like that of *O. pendulum* and probably the same relation of the leaf bundles to the bundles of the rhizome obtain as in *O. pendulum*. The difference in the venation has already been pointed out and the leaves differ also from those of *O. pendulum* in the fact that stomata are developed only upon the lower side. A feature of this species is the presence at the base of the leaves of very conspicuous multicellular branching hairs, quite different from those found in any other species of *Ophioglossum* (fig. 70, *C*). I find that the cells are much more elongated in my specimens than those figured in the account of the Ophioglossaceæ given in the Natürliche Pflanzenfamilien.*

## THE SPOROPHYTE OF BOTRYCHIUM.

There is much the same uncertainty as to the number of species of *Botrychium* that there is in *Ophioglossum*. Some of the species are exceedingly variable and their limits are difficult to determine. Bitter, in his account of the Ophioglossaceæ in Engler and Prantl's "Natürliche Pflanzenfamilien," states that there are sixteen species. Underwood (Underwood 1) says "about thirty," of which fifteen occur in the United States. Christensen in his Index Filicum recognizes thirty-four species.

Many of the species are very widespread, being most abundant in the North Temperate Zone. According to Christ (Christ 1), *B. lunaria* is not only widespread throughout the colder parts of the Northern Hemisphere, but occurs again in the corresponding regions of the Southern Hemisphere, having been reported from Patagonia, southern Australia, Tasmania, and New Zealand. A few species occur in the mountain regions of the tropics; thus *B. lanuginosum* is not uncommon in the highlands of Ceylon, where I collected it at an altitude of about 7,000 feet.

The simplest member of the genus is *Botrychium simplex*, a very variable species occurring at various places in North America, northern Asia, and Europe. Its smaller forms closely resemble a small *Ophioglossum* (fig. 72, *A*, *B*), except that the sporangia are more distinct. From this primitive type, obviously not very remote from *Ophioglossum*, there is an interesting series of forms leading up to the large species like *B. virginianum* and *B. silaifolium*. This latter species, which grows in the mountains of western North America, is perhaps the largest of the genus (plate 7). The plant is sometimes 2 feet high, the leaf nearly a foot in width, and the very large panicle of sporangia 6 inches or more in length.

In the evolution of the leaf in *Botrychium*, the sporangiophore shows a development parallel with that of the sterile leaf. In the forms of *B. simplex*, having an entirely undivided sterile lamina, the sporangiophore has the form of a simple spike with a single row of large sessile sporangia on either side (fig. 72, *A*) and very closely resembles a small *Ophioglossum*. As the sterile leaf segment becomes more and more dissected, there is a corresponding branching of the sporangiophore which,

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* Professor Bower, at the last meeting of the British Association for the Advancement of Science, read a paper on the Anatomy of *Ophioglossum palmatum*. This has not yet been published, but Professor Bower has kindly furnished me with an abstract of his paper from which the following extract is quoted.

"It was thought probable that *O. palmatum* (the only species of section *Cheiroglossa*) would share with the species named (*i. e.*, the species of *Ophioderma*) the character of a divided (leaf) trace, and material collected in Jamaica has shown that it does. The axis is much distended by parenchymatous storage tissue in pith and cortex, and as a consequence the meshes of the stele are transversely widened. From their margins right and left, but not quite simultaneously, arise two strap-shaped strands, which are thus widely apart in their origin. After subdivision into numerous smaller strands, these range themselves into two fan-like semicircles, which spread till their margins meet, forming the circle of strands of the petiole. A remarkable feature of the stock is the intrusion of roots into the bulky pith; this is especially obvious towards the base, where they pass out as thick mycorrhizic roots."

This behavior of the roots recalls that of the Marattiaceæ.

in the large forms of *B. virginianum* and *B. silaifolium*, forms a large panicle 15 centimeters or more in length and bears many hundred sporangia. Two types of leaf division may be noted, a pinnate form represented by *B. lunaria* and its allies, which are sometimes separated as a special genus *Eubotrychium*, and a ternate division which is found in all of the larger species. These larger species form a second section, *Phyllotrichium*. A third genus, *Osmundopteris*, has been proposed to include *Botrychium virginianum* and its allies. In most of the species of *Botrychium*



Fig. 71.

*Ophioglossum simplex*
Ridley (after Bower).
The fertile leaf has no
sterile lamina.

Fig. 72.—(A–D, after Luerssen.)

A, B. *Botrychium simplex* Hitchcock.   C, *B. ternatum* Swz.
D, *B. lunaria* (L.) Swz.   E, *B. virginianum* (L.) Swz.

the texture of the leaf is soft and fleshy, like that of *Ophioglossum*, but in *B. virgini-anum* it is thin and membranaceous, like that of many true ferns. In *B. lunaria* (fig. 72, *D*) the venation comes nearer to that of *Ophioglossum* than is the case in the larger species. The fan-shaped leaflets have no midrib and the veins branch dichotomously from the base of leaf, radiating from this point in the manner de-scribed as "cyclopterid." In the larger species the leaflets have a distinct midrib
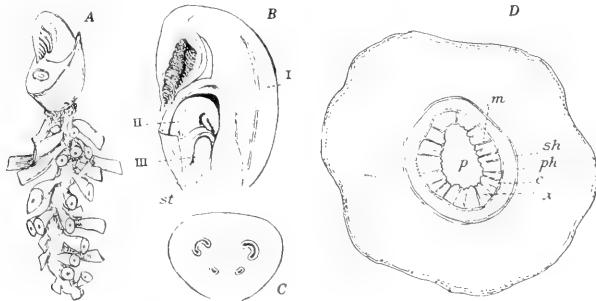


Fig. 73.

A. Rhizome of a strong plant of *Botrychium virginianum*. ×1. The base of the expanded leaf is cut away.
B. Section of the terminal bud. *st*, stem apex. I, II, III, the three youngest leaves. ×2.66.
C. Section of petiole, enlarged.
D. Section of rhizome; *p*, pith; *m*, medullary rays; *x*, xylem; *ph*, phloem; *c*, cambium; *sh*, endodermis. ×16.
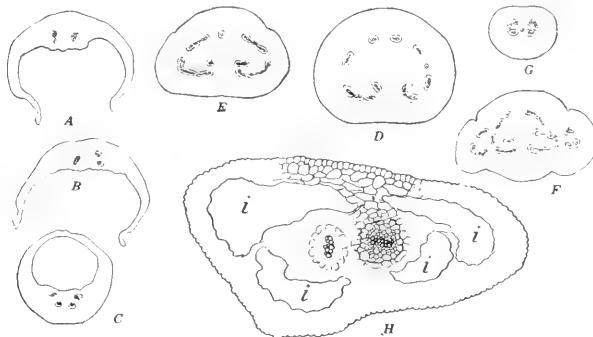


Fig. 74.

A-G. A series of sections of the sporophyll of *Botrychium lanuginosum* Wall. C is reversed in position. G, section of peduncle.
H. Section of the petiole of the cotyledon of *B. virginianum*. *i, i*, lacunæ.

and the forking veins are attached to this laterally (fig. 72, *E*). The type of *B. lunaria* is without question the more primitive, and were the ends of the veins united so as to inclose areoles there would be exactly the same type of venation as is found in *Ophioglossum*.

In *Eubotrychium* the anatomy of the leaf is almost exactly like that of *Ophio-glossum*, stomata being developed upon both sides of the leaf; the mesophyll is composed of absolutely similar cells throughout and the vascular bundles running

through the mesophyll do not project at the surface as veins. In the section *Phyllo-trichium*, where the sterile lamina is horizontal, stomata are developed only upon the lower surface. In these forms the mesophyll is more compact upon the upper side, but does not develop a distinct palisade layer. The epidermal cells may be simply elongated, e. g., *B. lanuginosum*, or they may be undulate, e. g., *B. virginianum*. The development of the stomata was examined in *B. lanuginosum* and the stoma was found to arise directly from an unmodified epidermal cell. The section *Eubo-trychium* shows a further approach to *Ophioglossum* in the character of its stipular sheath, which, as we have seen, forms a hood-like appendage at the base of the leaf, while in *B. virginianum* the stipular sheath is open in front, so that there are really two stipules. Moreover, the leaf in the latter is sharply bent over in the bud in a way to suggest somewhat the circinate vernation of the Marattiaceæ. For the most part the surface of the plant is quite smooth, as in *Ophioglossum*, but the sheaths
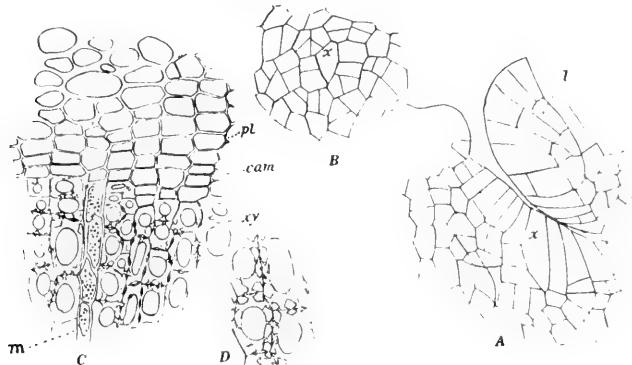


Fig. 75.

A. Stem apex of young sporophyte of *Botrychium virginianum*. *x*, apical cell; *l*, youngest leaf. ×200.
B. Cross-section of stem apex.
C. Part of vascular cylinder of stem. *pl*, phloem; *cam*, cambium; *xy*, xylem; *m*, medullary ray.
D. Some of the tracheids, more enlarged, showing bordered pits.

enveloping the young leaves, as well as the very young leaves themselves, are often hairy, and in a very few species, like *B. lanuginosum*, long and slender unicellular hairs are sparsely scattered over the surface of the adult leaf. The stem, as we have seen, is always an upright rhizome and usually unbranched, but adventitious buds may be developed (see Bruchmann 2). These adventitious buds may develop into lateral branches. In one specimen of *B. lanuginosum* examined by me there were two equal branches, presenting the appearance of a dichotomy, but a careful exam-ination indicated that the original apex had been destroyed and that these two branches were lateral shoots, which had probably arisen as adventitious buds. In *Eubotrychium* the leaves are arranged spirally upon the rhizome, as they are in *Euophioglossum*. This is also true of certain members of the section *Phyllotrichium*, but others, e. g., *B. ternatum*, are arranged in two rows, thus suggesting the dorsi-ventral arrangement found in *Helminthostachys*.

The structure of the adult stem does not differ, except in size, from that of the younger plants already described. In *Eubotrychium* the cambium is absent and apparently there is no secondary increase in diameter, but all of the larger species of *Phyllotrichium* show a greater or less development of the cambium, which we have

noted in the bundle of *B. virginianum*. The position of the sporangiophore is some-what different in different species. In *Eubotrychium* it is attached, as a rule, close to the junction of the sterile lamina and the petiole, very much as it is in *Euophioglossum*. In *B. ternatum* and *B. obliquum* the long peduncle is inserted very much further down, sometimes almost at the level of the ground, so that at first sight the spor-angiophore seems to be quite independent of the sterile leaf. In *B. virginianum*
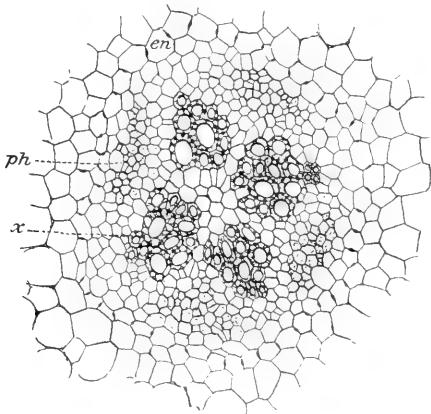


FIG. 76.

Tetrarch root of *Botrychium virginianum* en, endodermis; x, xylem; ph, phloem.
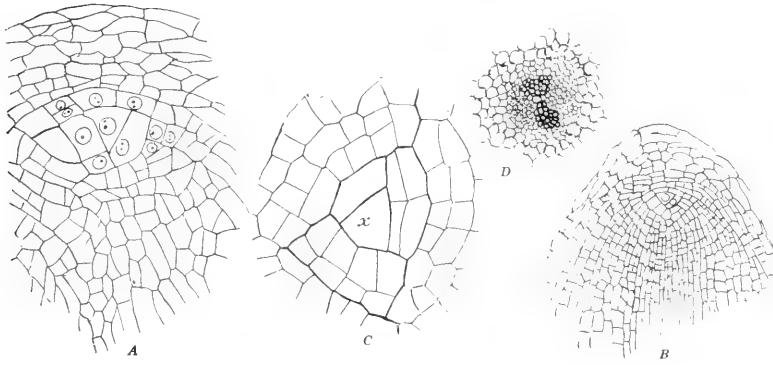


FIG. 77.

A. Apex of primary root of *Botrychium virginianum*. ×200.
B. Apex of second root. ×125.
C. Apex of large root, transverse section.
D. Diarch vascular bundle of the primary root.

and *B. lanuginosum* it is inserted close to the base of the sterile lamina or even above its base, and in the latter species there may even be sometimes a second spor-angiophore developed on the fertile leaf above the insertion of the primary one.

Certain species show various monstrosities, this being particularly the case with *B. lunaria*. In such forms certain of the segments of the sterile lamina may bear sporangia. A number of these curious forms are figured by Goebel (Goebel **2**).

In fig. 73, *A*, is shown the terminal bud of a strong plant of *B. virginianum*. The base of the oldest leaf is cut away so as to show the young leaf for the next year, which is seen to be strongly bent over, and the young leaf segments are rolled inward in a way that very strongly reminds one of the young leaves of the typical ferns. The young leaf is covered with hairs. The roots are thick and fleshy, probably one corresponding to each leaf, although this is not always easy to determine. They are much contracted at the point of insertion. The roots branch freely, the branching being monopodial, thus resembling *Ophioglossum pendulum*. An examination of the section of the bud shown in fig. 73 shows that the young leaf, which is to unfold the next year, is already well advanced and the formation of the young sporangia has just begun. The sporangiophore is also evident upon the leaf for the following year, and the leaf which is to unfold in the third year can be recognized, but its parts are not yet differentiated.

While the roots branch freely in *B. virginianum*, this is much less marked in *B. lunaria*. In large plants of *B. lanuginosum* which were examined somewhat carefully it was found that the roots appeared to be quite unbranched, although equaling those of *B. virginianum* in diameter and showing, like them, a similar tetrarch structure of the vascular cylinder. A more careful examination showed, however, a few very short branches on some of the roots, but these were rare. It may be that the rudiments of these lateral roots are formed, but that they do not develop, and in this respect there is a certain resemblance to the condition which obtains in *Helminthostachys*.

The anatomy of the roots does not differ from that of the younger sporophyte already described, except that in the larger specimens the roots are regularly tetrarch, while in *Eubotrychium* they are permanently diarch.

The earlier roots, as in *Ophioglossum*, have a tetrahedral apical cell which shows a more regular segmentation than is found in the later roots and in these earlier roots, at least in *B. virginianum*, there seems little question that the tissue of the root cap is derived entirely from the outer segments of the apical cell. In the larger roots, taken from the older sporophyte, this regularity is not so marked and it is probable, although this could not be determined absolutely, that the lateral segments of the apical cell may also contribute to the tissues of the root cap. The regularity of the segmentation is still less marked in the later roots of *B. obliquum*. A slight development of periderm may be seen in the older roots, but this does not form a continuous layer, being developed only in irregular patches. The secondary increase in diameter of the roots is apparently due almost entirely to simple enlargement of the cortical cells.

### THE SPOROPHYTE OF HELMINTHOSTACHYS.

While *Helminthostachys* differs very much in habit from the other Ophioglossaceæ, it nevertheless shows that it is unmistakably related to them and especially to *Botrychium*. The dorsiventral prostrate rhizome with the ample palmately divided leaves give it a very individual aspect, but, as is shown from a study of the early history of the sporophyte and the gametophyte, there is no question that it should be associated with the Ophioglossaceæ. As yet but a single species is generally recognized, which grows throughout the Indo-Malayan region, extending from the Himalayas through the Malay archipelago to northern Australia (plate 8).

The anatomical details of the adult plant have been very thoroughly investigated by Farmer and Freeman (Farmer **2**), and from a comparison of the structure of the adult sporophyte with that of the younger stages it is clear that the essential

characters are established at a comparatively early period. The rhizome (fig. 79) has the leaves arranged in two rows on the dorsal side, somewhat as in *Ophioglossum pendulum*, but the leaves are less crowded and the internodes are evident, although less marked than is often the case in the very young sporophyte. The youngest leaves, still inclosed in the stipular sheath of the next older one, are conspicuous as blunt projections, situated between the apex of the rhizome and the first expanded leaf. As the leaf breaks through the stipular sheath of the next older one the sheath is split into two stipule-like flaps, which much resemble superficially the stipules of the Marattiaceæ. From near the base of the leaves on the flanks of the rhizome and belonging to the ventral portion of the stem there arise the thick fleshy



FIG. 78.

A. Sporophyll of *Helminthostachys*, somewhat reduced.
B. Base of sporangiophore, about natural size.
C. Stoma.  ×200.

D. Four sections of the sporophyll    Fig. 3 passes through the base of the spike, *sp*; 4, section of peduncle of spike.

roots, which resemble those of the other Ophioglossaceæ. According to Prantl (Prantl 1) and Farmer (Farmer 2), the roots bear no definite relation to the leaf, and the number as a rule somewhat exceeds those of the leaves. The roots branch monopodially, as in the larger species of *Botrychium*, but Farmer states that the lateral rudiments often prove abortive, or fall away at an early period, so that the roots are apparently unbranched. This behavior recalls the condition found in *Botrychium lanuginosum*.

The leaves are very different in general aspect from those of the other Ophioglossaceæ, but are really not very different in their method of branching from the ternate forms of *Botrychium*. There are still traces of the three primary divisions of the first leaves which undergo subsequent divisions, so that the leaf has a palmate form (plate 8, *B*). To a certain extent, however, these divisions are the result of

unequal dichotomy, like that which results in the first divisions of the primary leaves. In large plants the leaves may reach a length of 30 or 40 centimeters. The petiole is nearly cylindrical at its base, but higher up becomes slightly winged at the margins, these wings passing into the lamina. A section of the petiole taken a little way below the insertion of the sterile lamina shows that the peduncle of the sporangiophore is recognizable for a long distance below the point at which it becomes free, its adaxial surface projecting as a rounded ridge between the wings on the margin of the petiole (fig. 78, $D$). In texture the leaves are firmer and more leathery than in any of the other Ophioglossaceæ, and in this respect, as well as in their venation, they closely resemble such Marattiaceæ as *Danæa* and *Angiopteris*.

The sporangiophore is to some extent intermediate in character between that of *Ophioglossum* and *Botrychium*. It is inserted near the junction of the lamina and petiole, but, as already stated, can be traced for some distance below the point where it joins the petiole. The fertile portion of the sporangiophore, which may reach a length of nearly 10 centimeters, is composed of crowded, short branches, upon
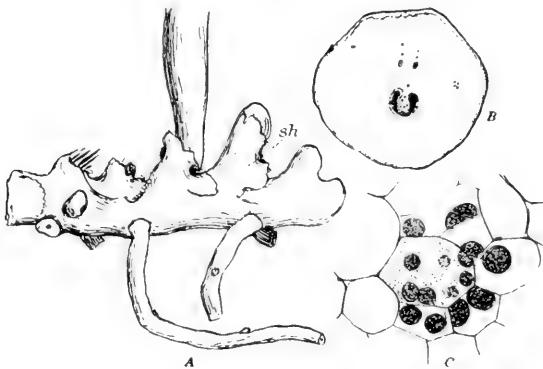


FIG. 79.

A. Rhizome of *Helminthostachys*. *sh*, stipular sheath.   ×1.5.
B. Section of rhizome. The dark spots are tannin sacs.
C. Tannin sacs from the rhizome.

which are borne groups of nearly globular sporangia much like those of *Botrychium*. The ends of the branches which bear the sporangia may be expanded into small leaf-like organs which, under exceptional conditions, look much like small leaves, upon which the individual sporangia are borne (see Bower 9, fig. 239). The stem in *Helminthostachys* almost always remains unbranched, except that there may be formed adventitious buds somewhat as in *Botrychium*. The origin of these buds, however, has not been investigated. The rhizome is rather fleshy in texture and may reach a diameter of about a centimeter. Usually but one leaf is fully developed at a time, but it is not uncommon to find two leaves produced the same season. Farmer's description and figures of the structure of the terminal bud show that it is essentially the same as we have found it to be in the younger plant, and, as has already been pointed out, it shows strong resemblances to the terminal bud in *Botrychium lunaria*.

The stomata (fig. 78, $C$) are confined to the lower surface of the leaf and are surrounded by a series of concentrically placed cells like those around the stoma in *Ophioglossum pendulum*. They are much smaller in size, however, and in this

respect may better be compared to the stomata of *Danæa*, one of the Marattia-
ceæ, which shows a similar concentric arrangement of the accessory cells. A
cross-section of the leaf shows that the mesophyll is better differentiated than in
*Botrychium*. There is a single compact layer of palisade cells below the epidermis,
and below these the mesophyll has very large intercellular spaces which communicate
with the stomata. In the anatomy of the leaf, therefore, as well as in its venation,
*Helminthostachys* more nearly resembles the Marattiaceæ than it does the other
Ophioglossaceæ. The section of the petiole is not unlike that of *Ophioglossum
pendulum* (figure 78, *D*). A section made near the base of the petiole is almost
circular in outline and there is a ring of separate bundles. On the adaxial side,
however, two other bundles are seen inside the outer circle. Farmer and Freeman
(Farmer **2**) state that the number of bundles is about seven or eight, but the
larger specimens which I have examined show a greater number. If a section of
the petiole is examined higher up it no longer appears circular, but is slightly
lobed, these lobes corresponding to the bases of the three divisions of the sterile
lamina, and on the adaxial side may be seen a fourth lobe which marks the position
of the peduncle of the sporangiophore. In this upper region of the petiole the
bundles become more or less coalescent, but two adaxial bundles remain quite
distinct and are continued upward into the spike. The structure of the bundles is
concentric and the general appearance of the section is quite similar to that found
in the larger species of *Botrychium*.

The anatomy of the stem in the older sporophyte does not differ in any partic-
ular, except in the greater size of its parts, from that of the sporophytes already
described. The vascular cylinder forms a hollow tube with narrow leaf gaps above,
but quite solid on its ventral face. Farmer states that there is both an outer and an
inner endodermis and that wood is formed inside of the protoxylem, so that the
bundle is mesarch.

Reference has already been made to the invaginated canals communicating with
the stipular cavities and first described by Gwynne-Vaughn (Gwynne-Vaughn **1**).
In connection with these he describes slight projections of tissue from the vascular
cylinder, which are surrounded by the endodermis. These projections he thinks
may perhaps be the vestiges of buds which formed at the axils of the leaves and
never developed. Whether this explanation is the correct one or not there is no
means of determining. Farmer found in the older plants that the roots were prevail-
ingly hexarch, ranging from pentarch to heptarch, and that tetrarch roots were rare.

The endodermis in the bundles of the later roots is much less distinct than it is
in the primary root, where it is very easily demonstrated. Within the endodermis
is a layer of pericycle cells, separating the endodermis from the radially arranged
masses of xylem and phloem. Farmer states that the apical cell of the root appears
triangular in section (presumably in longitudinal section), but that it is very often
destroyed and as a result of the proliferation of the adjacent tissue an area of cells
results recalling the condition obtaining in the Marattiaceæ. He also remarks that
most of the roots that he examined showed this abnormal condition.

I made sections of a number of roots taken from young sporophytes where the
root tips appeared to be quite normal. The result was rather interesting. In the
early roots there was usually present a tetrahedral apical cell, very much like that
of *Botrychium* and showing a pretty regular segmentation (fig. 80, *C*). The seg-
ments cut off from the apical cell are large, and even in the early roots it is pretty
certain that the root cap owes its origin, in part at least, to the outer cells of the lateral
segments. Cross-sections of the apical cell show the same triangular form as longi-
tudinal sections and the divisions in the segments of the apical cell seem to be very

much as they are in *Botrychium*. In one small root, however (fig. 80, *D*), the apex was occupied by three cells which together formed a triangular group, but these cells were of about equal size and it is not at all certain that the triangular cell *x* was really the apical cell.

In longitudinal sections of the larger roots there is a close approximation of the condition found in the Marattiaceæ. There is probably still a single apical cell, as there is in the early roots of the Marattiaceæ, but this cell is somewhat truncate below (fig. 53, *B*) and in a cross-section, taken from a tetrarch root, a single apical cell was not clearly to be distinguished. The lateral segments are very large and
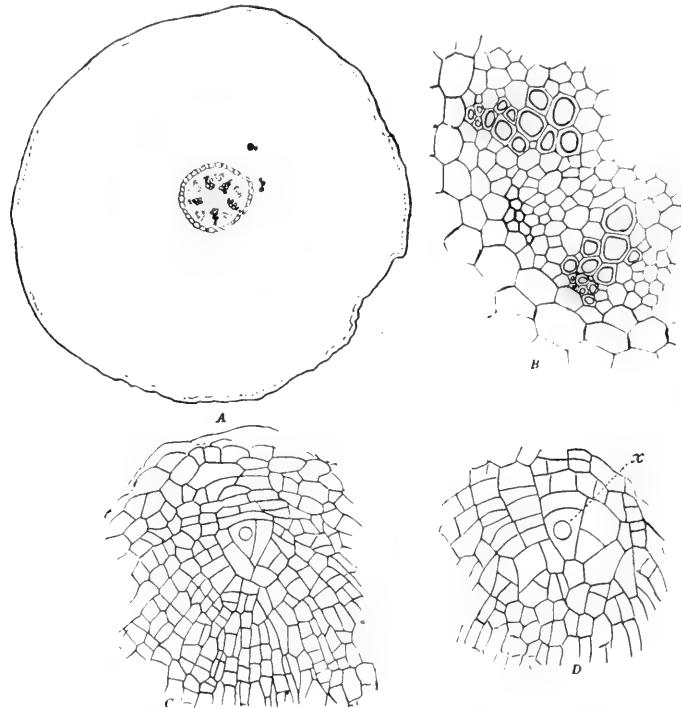


Fig. 80.

A. Section of pentarch root of *Helminthostachys*. ×20.　C. Root of young sporophyte, with tetrahedral apical cell. ×150.
B. Part of the vascular bundle. ×150.　　　　　　　D. Root of an older sporophyte, with truncate apical cell, x.

there are cut off from them cells which contribute to the root cap. In short, the apical growth in the later roots of *Helminthostachys* is evidently more like that of *Danæa*, for example, than it is like *Botrychium*.

### THE SPORANGIOPHORE OF THE OPHIOGLOSSALES.

In all of the Ophioglossaceæ the sporophyll, as we have seen, consists of two parts, the fertile spike (or sporangiophore) and the sterile lamina. There is very great diversity of opinion as to the real nature of the sporangiophore. The earlier views were strongly influenced by the old theory that the sporangial parts were

secondary modifications of an originally sterile leaf. At present it is the belief of most students of the ferns that the fertile structures of the sporophyll are older than the sterile ones, and this has led to a quite different interpretation of the real nature of the fertile segments.

Bower (Bower **9**) has treated at length the different theories that have been proposed to explain the nature of the fertile spike in the Ophioglossaceæ. We can only refer briefly to some of the more important of these. Mettenius (Mettenius **1**) considered that the two parts of the leaf were of equal value, but he gives no data as to their origin. Later writers, e. g., Holle (Holle **1**), Goebel (Goebel **1**), and very recently Chrysler (Chrysler **1**), consider the fertile spike to be the equivalent of the fertile pinnæ of such a fern as *Aneimia*. Holle and Chrysler think that the single median spike is the equivalent of two united pinnæ. Goebel thinks that the spike represents a single pinna which has arisen in a median position instead of upon the margin. Chrysler's results are based mainly upon a study of the distribution of the vascular bundles in *Botrychium*. His results, however, do not take into account the early development of the sporangiophore and its relation to the primordium of the very young fertile leaf.

Bower, who has made the most complete study of the development of the spore-bearing parts in the Ophioglossaceæ that has yet been published, concluded in his earlier work that the spike of *Ophioglossum* is morphologically equivalent to the single sporangium of *Lycopodium*. In this view he was supported by Strasburger and Celakovsky (Celakovsky **1**). More recently, however, he has modified his view (Bower **9**) and concludes that the sporangial spike is a distinct organ, for which he suggests the name "sporangiophore." This later interpretation we believe to be the correct one.

In *Ophioglossum* the sporangiophore develops a peduncle which is often very much longer than the sterile portion of the leaf and forms much the most conspicuous portion of the sporophyll (fig. 55). The fertile portion of the sporangiophore is a flattened spike along whose margins the two rows of large sporangia are borne. These project very little, the cavity of the sporangium being deeply sunk in the tissue of the spike and covered by several layers of cells forming the outer wall of the sporangium. The number of the sporangia in a spike may be only six or seven in some of the small forms of *Euophioglossum*, or it may be very large, as it is in the great spikes of *O. pendulum*.

In *O. pendulum* and *O. intermedium* the spike is more flattened than it is in the species of *Euophioglossum* and the central sterile portion is wider in proportion. Stomata are almost entirely absent from the epidermis of the spike in *O. pendulum*, although occasionally a few are found in the central region. In *O. intermedium* they are more numerous than in *O. pendulum*, but much less abundant than in the species of *Euophioglossum*. In the latter they may occur even upon the epidermis of the wall of the sporangium. The most marked exception in the position of the sporangiophore is seen in the section *Cheiroglossa* (plate 5). In this form there are several sporangiophores developed upon the leaf. Although these are apparently marginal in position, it is claimed by Bower that they are really always developed upon the adaxial surface of the leaf. Some of the smaller forms may show a single spike which occupies a central position, very much like that of *O. pendulum* (plate 5, 3).

In some of the simpler forms of *Botrychium simplex* the sporangiophore is very much like that of a small *Ophioglossum*, except that the individual sporangia are more distinct, although the base of the sporangium is coherent with the margin of the sporangial spike. In all of the other species of *Botrychium*, however, the sporangiophore branches, the degree of branching following very closely that of the

sterile lamina. In the larger species, like *B. virginianum*, many hundred sporangia may be developed upon a single sporangiophore. In these larger and more specialized forms the sporangium is usually smaller and is better differentiated, always having a more or less distinct pedicel.

The form of the sporangiophore in *Helminthostachys* is to some extent intermediate between that of *Ophioglossum* and *Botrychium*, but on the whole comes nearer to *Botrychium*. The sporangia are densely crowded along the flanks of the spike, thus forming two rows somewhat as in *Ophioglossum*, but they are very much more crowded and are formed in groups or synangia which project free above the surface of the young spike. The sporangia do not arise singly, but are borne upon short branches or secondary sporangiophores which suggest the lateral branches of the sporangiophore in *Botrychium*, but are far less regularly disposed. By the further growth of the sporangia the axis of the spike is entirely concealed and a casual examination would indicate that the sporangia were formed equally at all points of its surface, but a study of the development shows that they are lateral in structure, as they are in *Ophioglossum*.

### THE DEVELOPMENT OF THE SPORANGIOPHORE.

The sporangiophore in both *Ophioglossum* and *Botrychium* becomes visible at a very early period. In *Ophioglossum moluccanum* I have found that the sporangiophore can be recognized even earlier than Bower has stated for *O. vulgatum* and *O. reticulatum*. A median section of a very young fertile leaf in *O. moluccanum* is shown in fig. 56, *D*. The apex of the leaf has scarcely become free and the apex, as well as nearly the whole of the adaxial surface, is made up of large columnar meristem cells, of which it is difficult to say that any one is the single apical cell of the leaf. The leaf trace is already evident, passing into the base of the young leaf, which is strongly inclined forward so that the real apex of the leaf is not directed vertically upward, but is on the adaxial side of the leaf rudiment. The apex of the young sporangiophore arises from the tissue immediately below the apex of the sterile segment, and the whole of the adaxial portion of the leaf below the apex of the young sporangiophore may be said to belong to the latter and not to the sterile portion of the leaf. There is thus practically a dichotomy of the apex of the young sporophyll, and the two branches (viz, the sterile segment and the sporangiophore) are structures of equal rank. It would thus appear that the old view put forward by Mettenius of two equal branches of the fertile leaf is probably the correct one. It is probable that younger stages than those figured by Bower for *O. vulgatum* and *O. reticulatum* would show the same condition of affairs that we pointed out in



FIG. 81.

A. Longitudinal section of young sporophyll of *Ophioglossum moluccanum*, still inclosed in the stipular sheath, *sh*   ×50.
B. Apex of sterile lamina.  *sp*, sporangiophore.
C. Apex of sporangiophore.  ×125.
D. Transverse section of a very young sporophyll, showing dichotomy of apex.  ×125.

O. *moluccanum* and O. *pendulum*, and the conclusion that the young sporangiophore is an outgrowth of the sterile part of the leaf is the result of the subsequent rapid growth of the upper sterile portion of the leaf, which thus carries its apex far beyond the apex of the young spike.

The young sporophyll in O. *pendulum* (fig. 64, E) resembles that of O. *moluccanum*, except that all the parts are much larger. The apex of the young sporangiophore appears upon the adaxial side of the very broadly conical young sporophyll.

Bruchmann's studies on *Botrychium lunaria* indicate that in this species the separation of the fertile and sterile segments of the sporophyll is the result of a dichotomy of the apex of the sporophyll at a very early stage in its development.
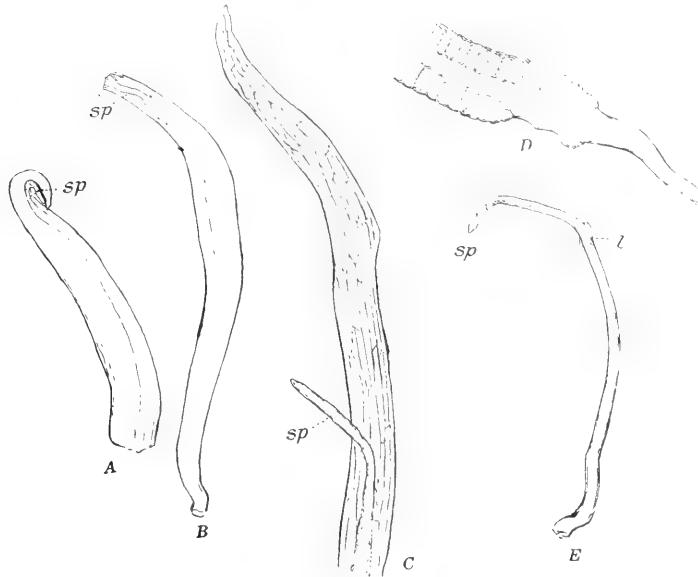


FIG. 82.

A. Young sporophyll of *Ophioglossum pendulum*. *sp*, the sporangiophore. ×2.
B. An older sporophyll. ×1.
C. A still older stage, showing venation of lamina.
D. Base of a full-grown sporangiophore.
E. Sporophyll with rudimentary lamina, *l*.

The sporophyll, however, is larger at this time than is the case in *Ophioglossum moluccanum* (Bruchmann 2, fig. 57).

The earliest stages in the development of the sporangiophore have not been studied in *Helminthostachys*. Bower says (Bower 9, page 455):

"The origin of it is similiar (i. e., to *Ophioglossum*) and its early stages not unlike. It appears at first as an outgrowth on the adaxial side of the sterile frond, and it is curved over while young so that the actual apex is pointed downward."

In the latter respect it might very well be compared to the strongly bent-over sporangiophore of *Botrychium virginianum*.

After the separation of the fertile and sterile portions of the sporophyll the sterile segment at first grows much more rapidly than the sporangiophore and soon extends beyond it, so that the sporangiophore has the appearance of being merely an adaxial appendage of the sporophyll. These changes in the relative importance of the points can be very well shown by a study of the very large sporophylls of *O. pendulum*. In this species the young leaf emerges from the sheath while it is still in a very early stage of development. These young leaves (fig. 82) have a very thick, fleshy leaf base, ending in the small pointed lamina, bent over and almost hiding the young sporangiophore which is attached to its inner surface. This curved-over form of the young sporophyll differs from that of *Euophioglossum*, where the young leaf is straight, and in this respect there is a suggestion of *Botrychium* or of the true ferns. The young spike at this stage is almost equal in length to the sterile lamina.

If the very young sporophyll of *Ophioglossum moluccanum* is examined, it will be seen that the vascular bundle leaving it passes downward and joins a young root trace. Very soon after the bundle enters the young leaf it divides into two branches, one of which passes into the base of the spike and the other and larger one into the sterile section of the sporophyll. This latter bundle now divides again into two, one of which passes to the dorsal side of the sterile segment while the other is turned toward the ventral side of the leaf, which probably represents tissue belonging to the sporangiophore. In the cross-sections of older leaves the petiole, which includes the completely fused base or peduncle of the sporangiophore, shows three distinct bundles, a large abaxial one and two smaller adaxial bundles. The former remains undivided for some distance above the base of the insertion of the peduncle of the sporangiophore. The two adaxial bundles divide into several branches, this beginning just below the point where the lamina of the sterile leaf becomes differentiated from the petiole. The lamina has its edges infolded so that it has a conical form, open in front and inclosing the young spike (figs. 56, 57). The successive branching of the two adaxial bundles follows in quick succession, and the outer bundles contribute to the veins of the lamina; the inner ones pass upward into the peduncle of the spike.

In a previous paper (Campbell 7) the conclusion was reached from the study of a series of sections at different heights, taken from the full-grown sporophyll, that the two adaxial bundles belonged exclusively to the spike. A study of the development of the young sporophyll shows that this statement is not entirely correct and that a considerable part of the vascular system of the sterile lamina owes its origin also to branches from the two adaxial bundles before the latter enter the peduncle of the sporangiophore. Of the two adaxial bundles, one represents the original bundle of the sporangiophore derived from the primary forking of the leaf trace; the other is derived secondarily from a subsequent division of the second branch of the leaf trace, this also giving rise to the large abaxial bundle of the petiole.

### THE DEVELOPMENT OF THE SPORANGIUM.

Much the most complete account of the development of the sporangium of the Ophioglossaceæ is that of Bower (Bower 5), who has made a very exhaustive examination of the development of the sporangium in all three genera. My own studies have been for the most part confined to *Ophioglossum pendulum*, *O. moluccanum*, and *Botrychium virginianum*. Burlingame (Burlingame 1) has more recently examined the development of the sporangium in a species of *Ophioglossum* from the Philippines, which was assumed to be *O. reticulatum*. Beers (Beers 1) has also published recently a somewhat incomplete account of the sporangium in *Hel-*

*minthostachys.* Through the kindness of Prof. L. L. Burlingame I have also had an opportunity to examine some of his preparations of *Ophioglossum reticulatum* and *Helminthostachys.*

An examination of both longitudinal and cross sections of the young spike in *O. moluccanum* (and this is true also of *O. pendulum*) shows that they agree entirely with Bower's account of the development in the species studied by him. There
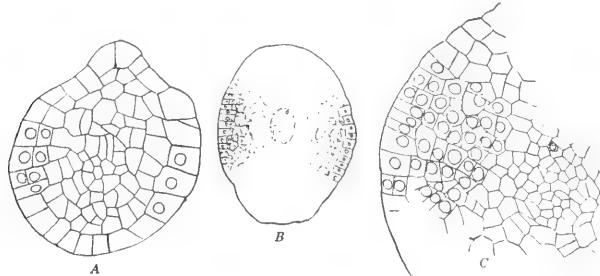


FIG. 83.

A. Transverse section of a very young sporangiophore of *Ophioglossum moluccanum.* ×150.
B. Section of an older sporangiophore. ×75. The shaded areas mark the "sporangiogenic" bands.
C. Part of B, more highly magnified. The nucleated cells mark the sporangial region.

seems to be no question that, as Bower first pointed out, there is formed a continuous band of tissue on each side of the sporangiophore, this band being the so-called "sporangiogenic" band, from which the individual sporangia are differentiated later. The sporangia arise from this sporangiogenic band at more or less definite intervals, these fertile areas being separated by bands of sterile tissue. In the sporangial areas periclinal walls are formed by which an inner mass of tissue, the
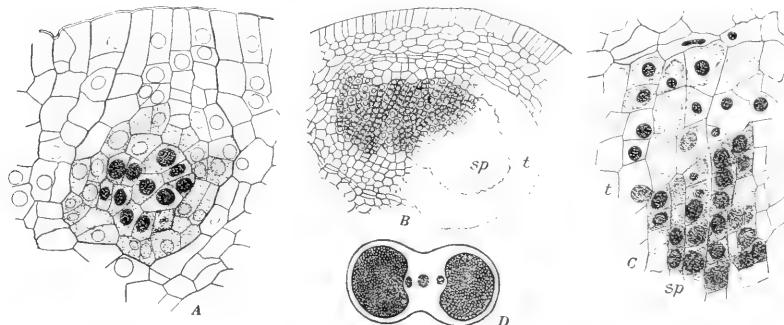


FIG. 84.

Development of the sporangium in *Ophioglossum pendulum.* The darkly shaded nuclei in A mark the archesporium; *t*, tapetum; *sp*, sporogenous tissue. A is a longitudinal section; B, a cross-section; C, part of B, more highly magnified; D, section of two mature sporangia. ×8.

archesporium, is separated from the outer cells which are to form the wall of the future sporangium. The inner cells constituting the archesporium later give rise to the masses of spores. Between the young sporangia there lie partitions or septa formed from the intervening sterile cells of the sporangiogenic band. The cell groups which form the archesporia and the sterile septa are derived from sister cells

8

of the original sporangiogenic bands. Bower thinks that all of the sporogenous tissue can not be traced back to a single primary archesporial cell, and that spore-bearing tissue is formed secondarily by periclinal divisions in the cells outside of the original archesporium. In some of the very young sporangiophores of *O. pendulum* there can sometimes be seen a single large epidermal cell which may possibly be the mother cell of a young sporangium, but it is very difficult to determine this point satisfactorily.

After the archesporium is differentiated there is a rapid division in its cells and there is thus formed a very large mass of cells, whose limits, however, are not always very clearly marked. Later on the contents of these cells become denser and are more easily distinguished from the surrounding sterile tissue.

The cells lying outside the archesporium divide rapidly both by longitudinal and transverse walls and give rise to the thick outer wall of the sporangium. In longitudinal sections through the sporangium two rows of cells may be seen extending from the mass of archesporial cells to the outside of the sporangium. These two rows of cells mark the point where the transverse cleft arises by which the sporangium opens at maturity. The outer cells derived from the archesporial complex
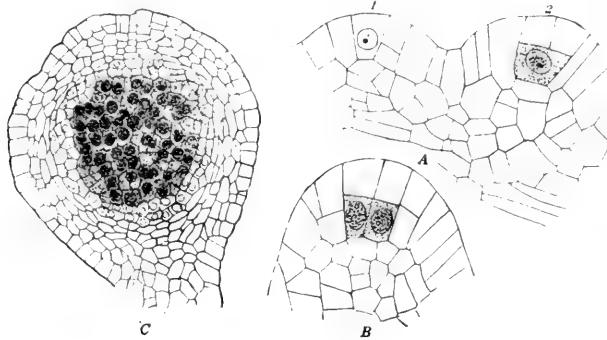


FIG. 85.

A, B. Young sporangia; C, an older sporangium of *Botrychium virginianum*. In C, the sporogenous tissue is shaded; the nucleated cells adjoining form the tapetum.

do not develop into spores, but constitute the tapetum, which later becomes disorganized and forms a sort of plasmodium extending among the growing spore mother cells, and is, no doubt, of great importance in the further development of these. Bower thought that some of the inner cells of the archesporial tissue contributed to this plasmodium, but a further study has led him to the conclusion that this is not the case and that all of the inner cells of the archesporium develop into spores. This view is confirmed by the recent paper of Burlingame (Burlingame 1), who investigated the development of the spores in *O. reticulatum*.

At maturity the sporangium opens by a transverse cleft whose position is already evident in the younger stages of the sporangium. As the cells shrink with the drying of the ripe sporangium the spores are crowded out through this cleft, but there is no special mechanism like the annulus found in the sporangia of the higher ferns which facilitate the dispersal of the spores.

Our knowledge of the development of the sporangium of *Botrychium* has been based largely upon the study of *B. lunaria*. I have investigated with some care the development in *B. virginianum*, which differs mainly from *B. lunaria* in the smaller

size of the sporangium and the fact that it has a well-marked though short pedicel. In the simplest forms of *Botrychium*, such as *B. simplex*, the sporangia are much larger and are sessile.

In *B. virginianum* (fig. 85) the development of the individual sporangia begins just about a year previous to their ripening, and if the plants are taken up about the time the spores are shed the earliest stages of the sporangia will be found in the leaf which is to expand the following season. At this time the sporangiophores in the larger specimens are thrice pinnate, and the youngest recognizable sporangia are borne at the tips of the branches. These young sporangia form slight elevations which become smaller as they approach the tip of the segments, and if an exact median section is made of one of these young sporangia it will be seen to have at its apex a large pyramidal cell with a truncate base. Holtzman (Holtzman 1), states that the whole sporangium may be traced back to a single cell and that the divisions at first are like those of a three-sided apical cell. I have not been able to satisfy myself as to the accuracy of this statement, but the youngest stages which I have been able to find would not forbid such an interpretation, although there seems no question that the basal part of the sporangium is derived, in part at least, from the surrounding tissue.
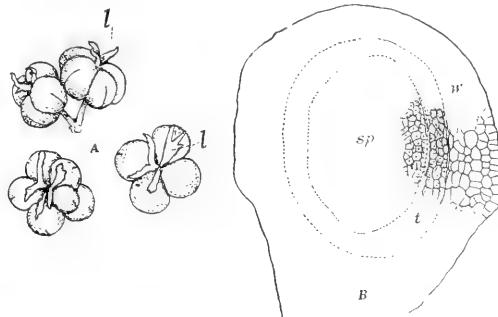


Fig. 86.

A. Sporangiophores of *Helminthostachys*. The two lower figures seen from above. *l*, sterile appendages. ×3.
B. Section of a young sporangium, showing sporogenous tissue, *sp;* tapetum, *t;* and wall, *w*. (From preparation made by Prof. L. L. Burlingame.)

The terminal cell of the sporangium is divided by a periclinal wall into a single inner cell which forms the archesporium, and an outer one which contributes to the wall of the sporangium. The outer cell rapidly divides and similar divisions occur in the archesporium. By active growth in the basal part of the sporangium it projects more and more until it assumes the form of a projecting globular body with a short stalk, thus differing strikingly from the deeply sunken sporangia of *Ophioglossum*. It is impossible to detect any definite succession of divisions in the archesporium, which ultimately becomes changed into a large globular mass of cells with the usual dense contents, separated from the outside of the sporangium by half a dozen or more layers of sterile cells. The cells immediately adjoining the sporogenous tissue constitute the tapetum. Bower states that the tapetum is exclusively derived from this outer sterile tissue, but it must be said that, in *Botrychium virginianum* at least, the limits between the sporogenous tissue and the tapetum are by no means easy to detect. The wall of the ripe sporangium has from four to six layers of cells and the dehiscence is by a vertical cleft, whose position may sometimes

be recognized in the young sporangium by cells arranged very much as they are in the sporangium of *Ophioglossum*. The stalk is traversed by a short vascular bundle which can first be recognized when the sporogenous cells are pretty well advanced in their development.

The sporangia of *Helminthostachys* on the whole more resemble those of *Botrychium* than those of *Ophioglossum*. In the very young sporangiophore there is developed a band of tissue on either side much like that in *Ophioglossum* (see Bower **9**, page 455). Transverse sections of the fertile spike show that the sporangiophores originate from certain groups of cells of this sporangiogenic band. These young sporangiophores, however, project above the surface and later form crowded projections which may undergo a greater or less degree of branching, so that the number of sporangia borne upon a single one of these secondary sporangiophores may be considerable. These sporangiophores become irregularly lobed and the final divisions of these develop into the sporangia in a manner which is very similar indeed to that of *Botrychium*, but the individual sporangia are usually less distinct. Bower's account of the further development may be quoted as follows:

"It has already been noted that the position and number of the sporangia which they bear are inconstant. In early stages it is impossible to distinguish the cells which will give rise to the sporangia, but from rather older stages it appears that the sporogenous groups, together with the superficial cells which cover them are referable in origin to the segmentation of a single superficial cell. Moreover, the first periclinal division of the cells defines the whole of the sporogenous tissue from the protective wall. As the sporangia grow older they project from the surface of the sporangiophore. The sporogenous mass increases rapidly in bulk, while the cells surrounding the sporogenous mass to the extent of several layers assume the character of a tapetum which gradually becomes disorganized; finally the sporogenous cells separate and divide into tetrads. As the sporangia approach maturity the upper part of the sporangiophore may grow out into an irregular rosette of laciniæ of vegetative tissue."

A study of the older sporangia of *Helminthostachys* shows that the tapetum is extraordinarily developed, constituting a very thick layer surrounding the mass of spores (fig. 86). The clusters of fully developed sporangia are often very regular in form (fig. 86, *A*), and remotely suggest the synangium of a Marattiaceous fern.

# Part II. THE MARATTIALES.

The members of the second order of the Eusporangiatæ—the Marattiales—resemble quite closely the typical Leptosporangiatæ. Both in the form and venation of the leaves and in the development of the sporangia upon their lower surface they recall the common ferns. But the structure of the sporangium is very different, and both in its structure and development the sporangium shows certain evident resemblances to that of the Ophioglossaceæ.

The Marattiaceous type is a very old one, and this fact lends a special interest to these few survivors of the ancient Paleozoic fern flora. Like the Ophioglossaceæ, the leaves of the Marattiaceæ are thick and fleshy in texture, but usually firmer than is the case in the Ophioglossaceæ. Some of the smaller species of *Danæa* are only 2 or 3 decimeters in height, but the larger species of *Marattia* and *Angiopteris* are among the largest of the ferns. The latter, which is common in the eastern tropics and extends to Australasia, is a noble fern whose great caudex, covered with the persistent leaf bases, is nearly as big as a barrel, and the gigantic leaves reach a length of 5 or 6 meters, with stalks almost as thick as a man's arm. In the dense, wet forests of Tjibodas in Java, for example, the development of this fern is especially luxuriant, and groups of them form one of the most striking features of this rich, tropical flora.

The Marattiaceæ frequent, for the most part, the moist, shady forests of the tropics, where they form a characteristic feature of the vegetation. Along the steep banks of moist, shady ravines or streams they sometimes occur in profusion.

The number of living Marattiaceæ[*] is small, some authorities recognizing only about thirty species. There is, however, a good deal of difference of opinion as to the number of species in some of the genera. This is especially true of *Angiopteris*, which many authorities consider to have only one single, extremely variable species, while others recognize many species. One of the genera, *Marattia*, is widespread throughout the tropics of both hemispheres, where there are about 25 species. (See Bitter 1, Christensen 1.) One of these, *M. douglasii*, extends to the Hawaiian Islands, where it is a common and conspicuous fern. *Angiopteris* is widespread throughout the tropics of the old world and reaches beyond the tropics into Australia and southern Japan. *Archangiopteris*, with a single species, *A. henryi*, is a recently discovered form occurring in southwestern China. *Kaulfussia*, also a monotypic species, occurs throughout the Indo-Malayan region and extends as far as the Philippines. The genus *Danæa* is exclusively neo-tropical and comprises about 20 to 25 species, most of which occur in the West Indies, northern South America, Central America, and Mexico. A sixth genus, *Macroglossum*, from Borneo, has recently been described (Copeland 1).

Of these, *Kaulfussia* is the most aberrant, differing markedly from the other genera in the form and venation of the leaves, as well as in the shape and position of the synangia. *Kaulfussia* and most species of *Danæa* have dorsiventral rhizomes, while the other genera have an upright, radially-constructed caudex.

The gametophyte, in all of the investigated species, is a large, dark-green, fleshy thallus, much resembling superficially such a liverwort as *Pellia*. The reproductive organs are very much like those of *Ophioglossum*, and chlorophyll is formed in abundance in the gametophyte. The large liverwort-like gametophyte

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[*] Christensen (1) recognizes 62 species of *Angiopteris*, 1 of *Archangiopteris*, 26 of *Danaea*, 1 of *Kaulfussia*, and 28 of *Marattia*.

and the character of the reproductive organs are marked indications of the primitive nature of these ancient ferns. The embryo also shows many points in common with that of the Ophioglossaceæ. As in the latter, the organs of the young sporophyte are all of epibasal origin and the vascular system in the young sporophyte belongs exclusively to the leaf and root, the stem being entirely destitute of a proper stele.

In *Danæa* a short suspensor is usually developed, but the other genera have as yet shown no examples of this.

The sporophyte is very much alike in its early development in all of the Marattiaceæ. The young plant consists at first mainly of the primary leaf and root, which are traversed by a single axial vascular strand as in *Ophioglossum moluccanum*, but a stem apex is developed at an early period, although it remains relatively inconspicuous.

*Kaulfussia*, *Danæa*, and *Archangiopteris* are all ferns of moderate size and comparatively simple structure; but *Marrattia*, *Angiopteris*, and *Macroglossum* are very large, and the arrangement of the vascular bundles in the stem becomes exceedingly complex, corresponding to the numerous bundles in the enormously developed leaves.

The form of the leaf in the Marattiaceæ ranges from a perfectly simple leaf in *Danæa simplicifolia* to the gigantic decompound leaves of *Angiopteris* and *Marattia*. Most of the species of *Danæa* (and this is true also of *Archangiopteris* and *Macroglossum*) have simply pinnate leaves, while in *Kaulfussia* the leaves are palmately divided into from three to seven leaflets, the larger leaves looking curiously like a horse-chestnut leaf in outline, whence the specific name. The ternate form, which is often found in *Kaulfussia*, recurs in the early leaves of the other genera and is sometimes retained in quite large leaves in *Marattia* (plate 12, B). This recurrence of the ternate leaf form suggests *Helminthostachys* and the ternate species of *Botrychium*. The leaves are usually quite smooth, but in the earlier stages there is sometimes a sparing development of hairs and scales. The latter are especially noticeable upon the leaves of the young sporophyte in *Danæa*.

The leaves are furnished at the base with very conspicuous fleshy stipules which remain adhering to the stem after the leaves fall away, and these leaf bases, with their attached stipules, more or less completely cover the surface of the stem. As the leaves fall away they leave a characteristic scar marked by the remains of the vascular bundles. The leaf base as well as the stalks of the leaflets shows a more or less marked enlargement, recalling the pulvinus which occurs so commonly in the Leguminosæ. It is at this point that the leaf stalk separates, the smaller divisions of the leaf often breaking away from the main or secondary rachis, in the same fashion as the main leaf stalk falls. In the large species of *Marattia* and *Angiopteris* this enlarged leaf base with the two thick, fleshy stipules curiously resembles in shape and size the hoof of a horse.

The leaves when young are coiled up in the same fashion as those of the typical ferns and their venation in general is decidedly fern-like, the ultimate veins being dichotomously branched and the venation very much like that of *Helminthostachys* or certain species of *Botrychium*. In *Kaulfussia*, however, the venation is reticulate and strikingly like that of the typical Dicotyledons. In its earlier stages, however, there is a marked resemblance to the venation of *Ophioglossum*.

The fleshy leaves do not, as a rule, show a very large development of mechanical tissues; but there is developed, especially in the larger leaves, a thick layer of hypodermal tissue, which is usually sclerenchyma, somewhat like that of the typical ferns, but may be thick-angled tissue or collenchyma, such as is common in many flowering plants. A marked anatomical feature is the presence of large mucilage ducts.

## I. THE GAMETOPHYTE.

The first account of the germination and development of the gametophyte in the Marattiaceæ was published by Luerssen (Luerssen 2), who studied the germination in *Marattia cicutæfolia* and in *Angiopteris*. Not long afterward the Dutch botanist, Jonkmann, published an account of the development of the prothallium in both *Marattia* and *Angiopteris*. His original paper (published in Dutch, but afterward translated into French) gives an extremely satisfactory account of the germination and development of the prothallium and reproductive organs (Jonkmann 1). Somewhat later he also published a preliminary account of the germination in *Kaulfussia*, but apparently the work was never published in full (Jonkmann 2). In 1892 Farmer (Farmer 1) described the gametophyte and embryo in *Angiopteris evecta* and in 1894 the writer (Campbell 3) gave an account of the prothallium and embryo in *Marattia douglasii* collected in Hawaii. Two years later Brebner (Brebner 2) described the prothallium and embryo in *Danæa simplicifolia*. In 1908 the writer published an account of the prothallium and young sporophyte of *Kaulfussia* (Campbell 9) and in 1909 a preliminary note was published in regard to *Danæa* (Campbell, 10).

The following account of the germination is based mainly upon the work of Jonkmann. I have, however, examined the early germination stages in *Marattia douglasii* for comparison with Jonkmann's account, and, so far as my experiments went, it agrees entirely with the species described by Jonkmann.

The ripe spores of the Marattiaceæ are small and may be either of the bilateral type or tetrahedral (radial). According to Jonkmann, the bilateral spores are much more abundant in *Marattia* than the radial spores, but in *Angiopteris* the radial spores predominate. The wall of the spore (see Jonkmann 1, pp. 203, 204) shows a differentiation into an inner membrane or endospore and a middle layer, the exospore, which is often found divided into layers. There is a very thin external coat, the perispore or epispore, which is generally thrown off in the early stages of germination or even before germination begins. All of the membranes except the epispore are colorless, while the latter is a more or less pronounced yellowish brown tint. The surface of the spore is roughened by small papillæ which arise from the exospore. The spores contain no chlorophyll, but there is a considerable amount of oil present, which appears as drops of varying size, and there are also other granular contents— starch and albuminous granules. The nucleus lies in the center of the spore and is connected with the peripheral protoplasm by delicate protoplasmic filaments. Germination begins quite promptly under favorable conditions and within about a week the spores, which hitherto were quite colorless, begin to show a greenish tint, due to the development of chlorophyll. Jonkmann states that the chlorophyll appears first as flocculent masses near the nucleus, but these apparently amorphous masses are really composed of very small, faintly tinted chromatophores, which lie between the large oil drops and rapidly increase in size and depth of color as germination proceeds, their number increasing by the usual division. The chloroplasts later become very conspicuous and are distributed in the periphery of the now very much enlarged spore, the outer membranes of which are ruptured so as to expose the endospore, containing the nucleus and numerous large and conspicuous chloroplasts. Starch granules are also to be seen in most cases.

The cell remains undivided until it has attained a size many times greater than that of the spore. The first division wall, which is formed about a month after the spores are sown, is transverse both in *Angiopteris* and *Marattia*, and, like that in the germinating spore of *Ophioglossum*, divides the primary cell into two nearly

equal parts (fig. 87, *A*). A rhizoid may be cut off either before or after this first transverse wall is developed, but frequently no rhizoids are formed until a much later period, as in *Ophioglossum*. The primary rhizoid, when present, is formed much as in the typical ferns, the papilla from which it develops being cut off from the larger cell, and it contains little or no chlorophyll. Each of the primary prothallial cells divides, in typical cases, by a longitudinal wall, so that the young gametophyte consists of four cells, arising quadrant-wise (fig. 88, *A*), and closely resembling corresponding stages in *Ophioglossum*, except for the absence of chlorophyll in the latter. Where the young plants are crowded or light is deficient, as for instance when the germination occurs within the sporangium, there is a tendency to the development of a filament, a phenomenon often met with also in the typical ferns.

Usually one of the upper pair of cells in the four-celled stage assumes the rôle of an apical cell, and for some time, as in the typical ferns, there is growth from a two-sided apical cell (fig. 88, *C*). As soon as the apical cell is established, it grows
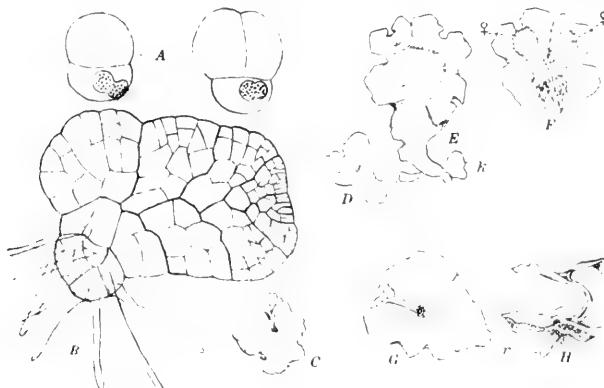


FIG. 87.

A. Two germinating spores of *Marattia fraxinea* Smith. ×200. The remains of the spore membrane can be seen.
B. Young gametophyte of same species. ×75. (A, B, after Jonkmann).
C. *Marattia sambucina* Blume. × 1.5.
D–H. *M. douglasii* Baker. D–F, × 1.5; G, H, ×3. G, H, show the young sporophyte.
D and E represent the same prothallium after an interval of about a year. F is the ventral view of E. *k*, adventitious bud.

very much as it does in such a liverwort as *Aneura* and produces a thallus of the same form and structure. But as the prothallium grows older a periclinal wall is formed in the apical cell, and in the outermost of the two cells thus produced there is a longitudinal wall dividing it into two equal cells, and from this time on it is impossible to recognize a single apical cell in the prothallium, the apex of which is occupied by a group of apparently similar marginal initial cells.

At first the prothallium has a spatulate form, but before the single apical cell is replaced by the group of marginal initials the outer cells of the younger segments grow more rapidly than the inner ones, so that they project beyond the apical cell, which thus comes to lie in a depression between the two lobes, and the familiar heart-shaped form so commonly found in the prothallium of most ferns is established. The marginal initial cells vary in number with the width of the depression in which they lie. In a horizontal section they appear oblong in form, but in the vertical sections made they have a semicircular outline (fig. 88, *D, E*).

Basal segments are cut off by a wall extending the whole depth of the prothallium and the segment thus cut off is divided at once by a horizontal wall into a dorsal and ventral cell of nearly equal size (fig. 88, $E, d, v$). Cell divisions are more active in the ventral segments, more manifestly so at some distance back from the apex. It is due to this more active cell division in the ventral segments that the strongly projecting cushion-like mass of tissue is formed upon the ventral surface of the prothallium, upon which the archegonia later make their appearance. The superficial cells of both surfaces of the prothallium have a thick cuticle which often makes it difficult to embed the prothallia without bad shrinkage. From the under side of the prothallium numerous rhizoids are developed, which in the case of *Marattia* and *Angiopteris* are unicellular and thin-walled, but in *Danæa* become divided into several cells. Sometimes there seems to be no definite apical growth in the early stages, but on the other hand Jonkmann states that both of the superior cells may function as apical cells, thus inaugurating the early dichotomy of the young prothallium, and even a third branch may arise from one of the inferior quadrants, which assumes the character of a third apical cell.

Among the leptosporangiate ferns, the forms which most nearly resemble the Marattiaceæ in the development of the gametophyte are the Osmundaceæ, especially
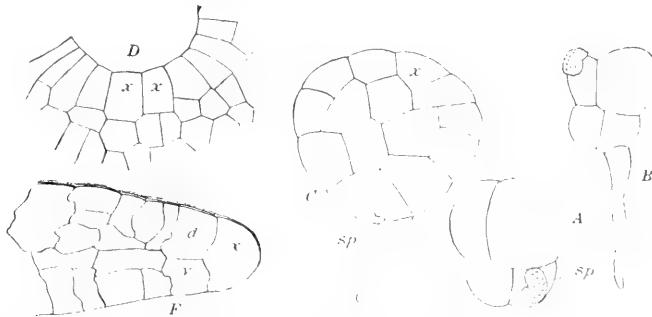


FIG. 88.

A–C. Three young gametophytes of *Angiopteris*. *sp*, spore membrane. A, ×200; B, C, ×160. (After Jonkmann.) D, horizontal, E, longitudinal, sections of the prothallium apex of *Marattia douglasii*. *x x*, initial cells.

the genus *Osmunda* (Campbell, Mosses and Ferns, 2d ed., pp. 347, 348). In *Osmunda* there is often a tendency also to the formation of a massive structure at an early stage in the development, due to the formation of cell divisions in three planes, so that the prothallium becomes from the first more than one cell thick. This tendency in the Marattiaceæ becomes much more pronounced as the gametophyte grows and very soon there is evident a very thick midrib, which often becomes exceedingly conspicuous in the older gametophyte, unlike the typical ferns, where the thickening of the prothallium is confined to the region which bears the archegonia. In the Marattiaceæ this thickening extends almost to the margin of the prothallium, so that it is only at the extreme edge that the prothallium shows but a single layer of cells. The very old prothallia in *Marattia* branch dichotomously (fig. 87, $E, F$), and the process is entirely similar to that found in many thallose liverworts. The original growing point becomes divided by the development of a median lobe, thus inaugurating two growing points, and the midrib back of the growing point forks in exactly the same way as in many liverworts.

Besides this dichotomous branching, it is not at all uncommon to have adventitious buds developed upon the margin of the thallus. These form small secondary

prothallia, which may be detached and become independent plants; or reproductive organs may be developed upon them, usually only antheridia, while they are still connected with the mother plant. The prothallia are very long-lived and apparently may grow indefinitely so long as the archegonia are not fecundated. I kept prothallia of *Marattia douglasii* for nearly two years, during which time they grew vigorously and finally reached a length of over 2 centimeters. At the end of two years there was no indication of the slightest decrease in their vigor.

The prothallia are monœcious, although it is not uncommon to find small prothallia which bear only antheridia. They are very deep green in color, which together with their more massive texture makes them easily distinguishable from the prothallia of the ordinary ferns, and they very often look more like such a liverwort as *Pellia* or *Anthoceros*. The prothallium of *Angiopteris* very closely resembles that of *Marattia*, but is often somewhat shorter. The difference in shape in the prothallia of the form studied by Jonkmann (*A. pruinosa* var. *hypoleuca*) and the Ceylonese form studied by Farmer and myself may be taken as an argument in favor of recognizing a specific difference between these two forms.

The archegonia in both *Marattia* and *Angiopteris*, so far as my own observations extend, are confined to the lower surface of the midrib. Jonkmann, however, states that occasionally he observed archegonia developed upon the upper surface as well. The antheridia, while more abundant upon the lower surface of the prothallium, are quite commonly met with also upon the upper surface and are not restricted to the midrib, but may be found quite near to the margin. The rhizoids in both *Angiopteris* and *Marattia* are probably always unicellular, and their walls, which are quite strongly cutinized, are dark brown in color.

FIG. 89.

Two gametophytes of *Angiopteris* with young sporophyte attached. *em*, embryo; *cot*, cotyledon. ×3.

## THE PROTHALLIUM OF KAULFUSSIA.*

The prothallia of *Kaulfussia* are usually much larger than those of either *Marattia* or *Angiopteris*. They are very massive, strongly resembling a *Pellia* or *Aneura*. The specimens described here were collected in Java in a small ravine near the foot of the volcanic mountain Salak. The youngest prothallia found were about 5 millimeters in length and, like the older ones, were decidedly elongated, with a deep sinus in front (fig. 90, *A*, *B*). There were a few antheridia occupying the forward part of the thick midrib, which is very largely developed in *Kaulfussia*, as it is in the other Marattiaceæ. The older prothallia are relatively somewhat broader and these larger ones usually bear archegonia. Only in a few cases were young antheridia found upon the prothallia with the archegonia. Whether this is always true could not be decided from the small number of prothallia found. The antheridia in *Kaulfussia* seem to be strictly confined to the lower surface of the midrib and occupy much the same position that the archegonia do. After the antheridia have matured and discharged the spermatozoids, archegonia arise in the same position in regard to the apex. Unlike most ferns, the walls of the empty antheridium do not become discolored, so that they are easily overlooked. Careful examination of the sections of the older prothallia, however, will almost always show the empty antheridia and it is probable that the prothallia are usually

* *Kaulfussia* Blume = *Christensenia* Maxon.

proterandrous and not diœcious, as might be supposed from a casual examination. The margins of the large prothallium are more or less irregularly lobed and it is not unlike that of *Osmunda*, but as in the other Marattiaceæ the wings of the prothallium are several cells in thickness near the midrib, and only at the extreme edge are they reduced to a single cell in thickness.

The full-grown prothallia are a centimeter or more in length and nearly as broad. One very large specimen was found (fig. 90, *F*). This bore a young sporophyte with two fully developed leaves, and the prothallium measured nearly 2.5 centimeters in length by 1.75 in extreme width, and was also very thick. A second archegonial cushion was present, but whether this was due to a forking of the original apex, such as not uncommonly occurs in *Marattia* and *Angiopteris*, was not determined, although this was very probably the case.
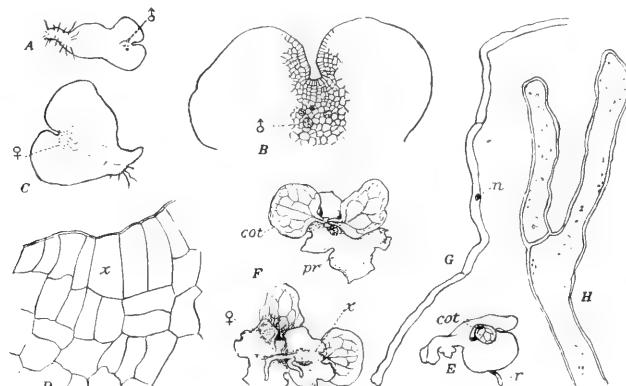


FIG. 90.—*Kaulfussia æsculifolia* Bl.

A. Young gametophyte. ×4.
B. Apex of A, showing the antheridia, ♂ ×20.
C. An older gametophyte, with archegonia, ♀. ×4.
D. Apical region of an older gametophyte; *x*, apical cell.
E. An older gametophyte, with young sporophyte attached. *cot*, cotyledon; *r*, root.
F. Two views of a large gametophyte, with attached sporophyte, natural size.
G. Rhizoid. ×50.
H. Apex of rhizoid, more highly magnified. A single nucleus only is present.

The rhizoids are stout and they have thick but quite colorless walls. They often show apparently transverse septa and the extremity is not infrequently forked (fig. 90, *H*). An examination of these rhizoids shows that these septa are not the result of true cell division, as only one nucleus can be found in the whole rhizoid. As the nucleus is large and conspicuous, and only one can be seen, it looks as if the formation of septa is secondary and not connected with cell division. The structure of the apex of the prothallium is exactly as in *Marattia* and *Angiopteris*. The apical meristem shows the usual row of marginal initial cells, of which one (fig. 90, *D*, *x*) is often somewhat larger than the others and may possibly represent a single apical cell.

## THE PROTHALLIUM OF DANÆA.

In July, 1908, the prothallia of three species of *Danæa* were collected in Jamaica. As the genus *Danæa* has received comparatively little attention, especially as regards the gametophyte, it seemed very desirable to secure as complete a series of the pro- thallia and young sporophytes as could be done, and to this end the trip was made to the West Indies. The genus *Danæa* is confined to the American tropics and comprises, according to Christensen, 26 species of extremely characteristic ferns. The type is evidently an old one, as some of the fossil Marattiaceæ are closely allied to the living genus *Danæa* and may possibly be referred to it. The only account of the prothallium hitherto published, so far as I am aware, is the paper of Brebner (Brebner **2**) on *D. simplicifolia*, a species from British Guiana.

In 1897 I collected a small number of specimens of prothallia of *Danæa*, prob- ably *D. jenmani*, and in the summer of 1908 the same locality was visited, as well as some others in the same district, and material of three species was secured. These



FIG. 91.

A. Three gametophytes of *Danæa jenmani* Underwood.  ×2.
B. Three young gametophytes of *D. jamaicensis* Underwood.  ×4.
C. Four large gametophytes of *D. jamaicensis*.  ×2.
C, 1, shows four groups of archegonia, ♀.

collections were all made in the vicinity of Cinchona, a mountain station at an eleva- tion of about 5,000 feet. As I have found in collecting other Marattiaceæ, the most favorable collecting ground for the prothallia is upon moist, clayey banks where the fruiting plants are growing. In the shady crevices in such positions a careful search will usually be rewarded by the discovery of numerous young plants, and with these there are often associated prothallia in various stages of development. Brebner described the prothallia of *D. simplicifolia* as being nearly circular in outline, but very few of the prothallia of the species collected by me showed this form; but they were usually decidedly elongated, sometimes very strongly so, and were, as a rule, very much larger than the specimens of *D. simplicifolia* described by Brebner.

The three species collected by me were *D. jamaicensis* Underwood, *D. jenmani* Underwood, and *D. elliptica* Smith. In all three species the larger prothallia are usually decidedly elongated and very irregular in outline, often showing conspicu- ous leaf-like marginal lobes, like those occurring in the prothallia of *Osmunda* and *Gleichenia*.

The germination of the spores and the early stages are quite unknown in *Danæa*, but probably resemble those of the other genera which have been studied. To judge from the younger stages collected, there is a good deal of variety of form, as is the case also in the other Marattiaceæ. The youngest specimens collected belonged to *D. jamaicensis*. These were very broad in outline and unsymmetrical, one wing of the prothallium being much better developed than the other (fig. 91, *B*).
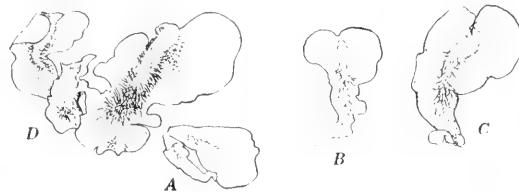


Fig. 92.—Four gametophytes of *D. elliptica* Smith. ×2. B–D, seen from below.

**Fig.** 92 shows some older specimens of *D. elliptica* which were greatly elongated, the very much attenuated posterior region being quite thin and delicate in texture, with no midrib and the archegonial cushion being confined to a small region just back of the growing point. The marginal region in these young prothallia was composed of a single layer of cells and a considerable portion was made up of but



FIG. 93.

A. Young gametophyte, probably developed from an adventitious bud of *Danæa jamaicensis*.
　×20. ♂ antheridia.
B. Large gametophyte of *D. elliptica*, with two sporophytes. ×2.
C. Multicellular rhizoid. ×100.
D. A cell of the rhizoid, showing nucleus, *n*. ×210.

two layers, while the mid region, where the antheridia and later the archegonia are developed, was not more than four to five cells in thickness. In general the prothallia of *Danæa* are more delicate in texture than those of the other Marattiaceæ that have been studied.

A midrib usually begins to form at an early period and in the older prothallia may become very conspicuous, sometimes reaching a thickness of eight to ten cells. The margin, as we have seen, is always more or less irregular in outline and often develops large leaf-like lobes which are particularly conspicuous in *D. jamaicensis* (fig. 91, *C*), but are noticeable also in the other species. The large prothallia, which are sometimes nearly 3 cm. in length, are often branched (fig. 92, *D*); the branching
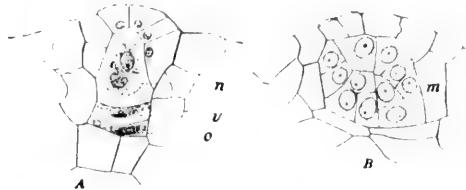


FIG. 94.

A, archegonium; B, antheridium of *Marattia douglasii*.  *n*, neck canal cell; *v*, ventral canal cell; *o*, egg; *m*, mantle cells.

is sometimes unmistakably a true dichotomy, but again it seems to be adventitious. In one large prothallium of *D. jamaicensis* of very irregular form, four groups of archegonia were present, widely separated from each other. It could not be certainly determined whether these arose from a repeated forking of the original apex or whether one or more of them was of adventitious origin (fig. 91, *C*).
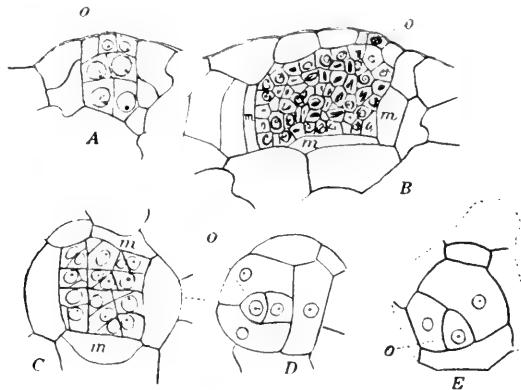


FIG. 95.

Antheridium of *Angiopteris*.  ×275.  *m*, mantle cells; *o*, operculum.
A, B. Longitudinal sections.  C. Transverse section.  D, E. Surface view.

The apex of the prothallium in the younger stages is usually indented by a sinus, but in the older ones the heart shape is almost completely lost and the apex may even project as a rounded protuberance, bearing the archegonia upon its lower surface, or it may be fan-shaped, with little or no indentation at the growing point.

The prothallium is the usual dark-green color found in the other Marattiaceæ, but as we have seen is rather more delicate in texture. Of the three species examined, *Danæa elliptica* resembles more nearly the prothallia of the other Marattiaceæ in the thickness of the central portion, which may show from eight to ten

layers of cells. The form and division of the apical cells is exactly the same as in the other Marattiaceæ. Stiff brown rhizoids are developed upon the lower surface and are mainly confined to the midrib, over which they may be evenly distributed, or there may be certain regions of considerable extent which are quite destitute of them. The rhizoids in all the species which have yet been examined are truly multicellular, as was correctly shown by Brebner to be the case in *D. simplicifolia*. The nuclei are not very large, but can be readily demonstrated.

The distribution of the reproductive organs is not always the same. Apparently the usual course of development is present here, the antheridia appearing first upon the lower side of the midrib and the adjacent points of the prothallium; later, nearer the apex and upon the lower side, the archegonia arise. Sometimes, however, as in the other forms, there is a more or less marked tendency to diœcism and some of the smaller prothallia seem to bear only archegonia or antheridia, and it is common to find antheridia developing exclusively upon the lateral lobes, which
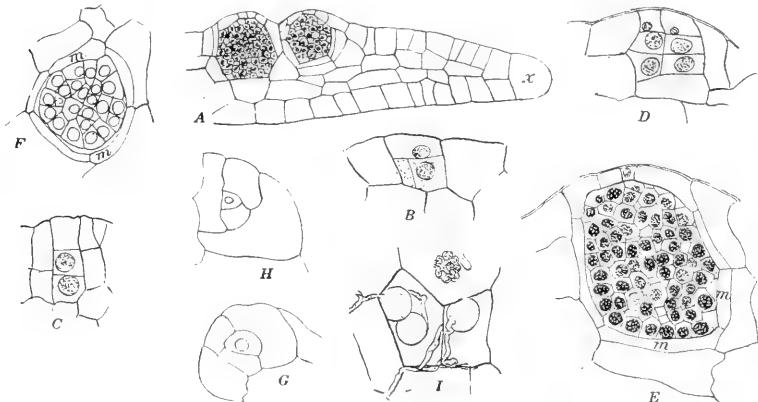


FIG. 96.—*Kaulfussia.*

A. Section of a young prothallium, showing two antheridia on the ventral side (reversed in the figure). ×80.
B–E. Longitudinal sections of antheridia.
F–H. Transverse sections. G, H are surface views showing the opercular cell. *m*, mantle cells. ×180.
I. Cell from interior of prothallium, showing the endophyte.

apparently never produce archegonia at all. Antheridia may also occur upon the upper surface, but this is not common, and in no cases were archegonia seen except upon the lower surface of the midrib. The reproductive organs are sometimes found in great numbers upon the older prothallia, the whole lower surface of the midrib being beset with archegonia. Quite as often, however, extensive areas along the midrib are quite sterile and the archegonia thus appear in patches, separated by sterile intervals.

### THE ENDOPHYTE OF THE MARATTIACEÆ.

In all of the Marattiaceæ that I have studied, an endophytic fungus very much like that which occurs in the prothallium of the Ophioglossaceæ has been found occupying the central cells of the prothallium in nearly all cases. The endophyte which infests the green prothallium of the Marattiaceæ, when compared with that found in the strictly saprophytic prothallia of the Ophioglossaceæ, shows some differences which are probably not without significance. The structure of the myce-

lium and its general behavior are so much like the form which occurs in *Ophioglossum* as to leave little room for doubt that the two forms are either identical or very closely related. The conidia (fig. 96, *I*), while occurring in the Marattiaceæ, are perhaps less frequent, but in form and structure are much like those of the endophyte of *Botrychium*. The most noticeable difference is the absence of the digestive cells, i. e., those that contain the varicose swollen mycelium. No evidences were found in the Marattiaceæ of the destruction of the fungus by the cells of the host and it is likely that the endophyte in these green prothallia is more nearly a true parasite than is the case in the saprophytic gametophytes of the Ophioglossaceæ. In the infested cells of the green gametophyte the starch and chromatophores are destroyed by the action of the endophyte, but the nucleus of the cell remains intact.

## THE SEXUAL ORGANS.

### THE ANTHERIDIUM.

The development of the antheridium, except for the details of spermatogenesis, was correctly described by Luerssen and Jonkmann for *Marattia* and *Angiopteris*. The other genera agree closely with these in the essential structure of the antheridium. The development of the antheridium in the Marattiaceæ agrees very closely indeed with that of *Ophioglossum*.
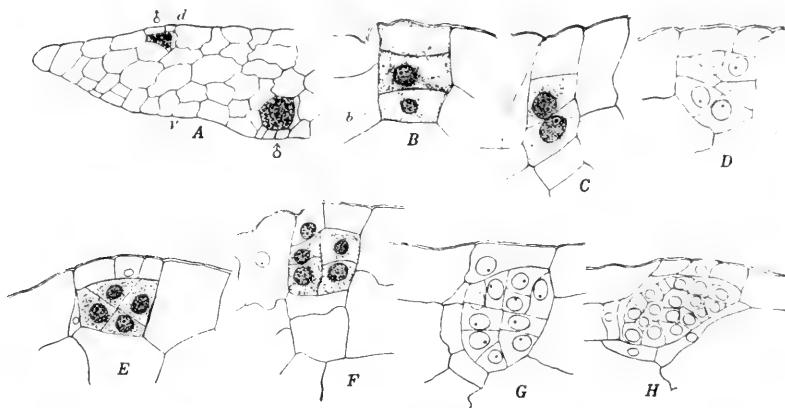


FIG. 97.—Development of the antheridium in *Danæa*.

A. Section of prothallium, bearing antheridia on both surfaces. ×80.
B–H. Longitudinal sections. ×300. E–H, *D. elliptica;* the others, *D. jamaicensis.*

The mother cell of the antheridium, as in *Ophioglossum*, divides first by a periclinal wall into an outer cell, the primary cover cell, and an inner one, from which the mass of spermatocytes is developed (figs. 96, 97). The mother cell of the antheridium shows much the same variation in form as that of *Ophioglossum*, sometimes being relatively broad and shallow and at other times deeper and narrower (fig. 96, *B*, *C*). The first division in the inner cell is usually transverse, but in the broader type of antheridium this first wall may be longitudinal. The primary wall is followed by a second one at right angles to it and the four cells thus formed are again divided so that there result eight nearly equal cells. The first wall occasionally is somewhat oblique, but even in such cases the regular quadrant and octant walls arise at right

angles to the primary division wall. The next divisions are, usually at least, anti-
clinals (fig. 97, G), but before long the periclinal walls also are developed and sub-
sequent divisions do not show any recognizable regularity in their sequence; there
seems to be a good deal of variation in this respect, even in the same species. The
number of cells ultimately formed varies a good deal, but the number of spermato-
cytes finally developed is probably never so great as that found in some of the
Ophioglossaceæ. *Kaulfussia*, both in the size of the antheridium and that of the
spermatozoids, approaches nearest to *Ophioglossum*. The number of spermatozoids
may reach several hundred, fifty or more being visible in a single section of a large
antheridium, and nearly or quite as many may sometimes be found in *Danæa*,
where, however, the spermatozoids are much smaller than in *Kaulfussia*.

In the cover cell the divisions are all anticlinal and horizontal sections or
surface views show that the successive walls are arranged spirally in a way suggesting
the segmentation of a three-sided apical cell. The last-formed wall cuts out a small,
nearly triangular cell, the opercular cell (fig. 95 *D, E, o*). In most cases, at least,
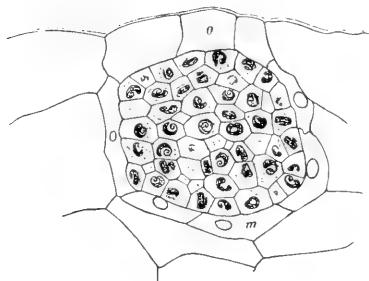


FIG. 98.

Ripe antheridium of *D. jamaicensis*. *m*, mantle cells.
*o*, opercular cell. ×350.
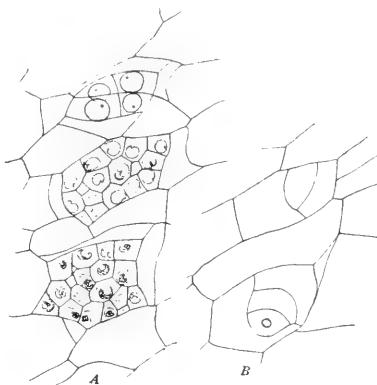
FIG. 99.

A. Transverse section of three young antheridia of *D. jamaicensis*.
B. Surface view of the two youngest ones. *o*, opercular cell. ×380.

this opercular cell is thrown off when the antheridium opens, leaving a small
aperture through which the spermatozoids are ejected. Surrounding the mass of
spermatocytes is a layer of mantle cells cut off from the adjacent cells of the prothal-
lium. These mantle cells, at the time of the opening of the antheridium, become
very much distended and project strongly into the cavity of the empty antheridium
(fig. 100, *B, m*). They no doubt play an important part in the dehiscence of the ripe
antheridium.

### SPERMATOGENESIS. (Plate 2, figs 38-44.)

Of the Marattiaceæ, *Kaulfussia* is the most satisfactory for studying the details
of spermatogenesis, owing to the much larger size of the spermatozoid. The
development of the spermatozoids agrees very closely in its details with that of
*Ophioglossum*. If the spermatocyte is examined just before the final division the
two blepharoplasts can be seen and the division of the cell into the two spermatocytes
proceeds very much as in *Ophioglossum*. After the final division the nucleus of the
spermatocyte appears coarsely granular and, as in other cases, no nucleolus can be
seen. In favorable cases the blepharoplast is visible as a round body, stained

9

rather strongly and lying near the nucleus. The blepharoplast soon takes on the curved form and becomes much extended and the cilia begin to develop from it before the nucleus has materially changed its shape.

The nucleus now becomes slightly pointed at one end and begins to stretch out so as to appear somewhat crescent-shaped, very much as in *Ophioglossum* and as it does in other ferns that have been described. With this change in the form of the nucleus, the blepharoplast becomes still more elongated and strongly colored and the cilia increase in length. The nucleus of the spermatozoid in *Kaulfussia* is less elongated than is usual in the ferns, and in this respect, as well as in its larger size, it more nearly resembles *Ophioglossum* than it does the other Marattiaceæ. The granular appearance of the nucleus is maintained until the spermatozoid is almost fully developed, when there seems to be a fusion of the chromosomes so that it appears almost homogeneous; this is accompanied by a noticeable diminution in the size of the nucleus. The nucleus occupies only the large posterior coil of the spermatozoid, while the anterior portion, which shows about two coils, is composed of the blepharoplast with probably a certain amount of other cytoplasmic matter.
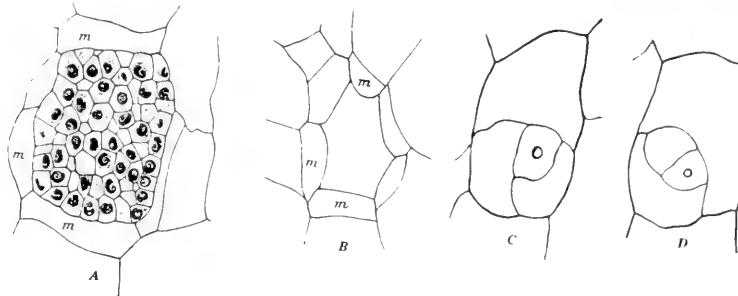


Fig. 100.
A. Cross-section of a ripe antheridium of *D. jamaicensis.*
B. Cross-section of an empty antheridium; *m,* mantle cells.
C-D. Surface views, showing opercular cell.
All figures × 350.

In *Marattia* and *Angiopteris* (plate 2, fig. 44) the nucleus of the spermatocyte becomes much more extended and the whole spermatozoid is more slender than in *Kaulfussia.* Indeed there is very little difference between the appearance of the spermatozoids of *Angiopteris* and *Marattia* and those of the typical leptosporangiate ferns. Some observations were made also upon *Danæa,* in which the spermatozoid is somewhat intermediate in character between that of *Angiopteris* and *Kaulfussia* (plate 2, figs. 42, 43). In size the spermatozoids are more like those of *Angiopteris,* but the nucleus is much less elongated and the general form of the spermatozoids is more like that of *Kaulfussia.*

### THE ARCHEGONIUM.

The archegonium in the Marattiaceæ, like the antheridium, very much resembles that of *Ophioglossum,* but the neck of the archegonium is even less developed than in the latter. Jonkmann (Jonkmann 1) has given a fairly complete account of the development in *Marattia* and *Angiopteris,* and Farmer (Farmer 1) has described and figured the archegonium of the latter genus.

Usually, at least, the archegonium is developed only upon the cushion of tissue back of the apex, the young archegonia arising in acropetal succession. Jonkmann

states, however, that he has found them also upon the upper surface of the prothallium, but none of the specimens I have examined have shown this. The mother cell of the young archegonium is scarcely distinguishable in form from the young antheridium and like it is first divided by a periclinal wall into an outer cover cell and an inner cell, the latter usually, but not always, divided subsequently into a central and a basal cell (figs. 101–103), as in *Ophioglossum* and in the typical ferns. Sometimes the mother cell of the archegonium is seen in transverse section to have been cut out very much as the axial row of cells arises in the archegonium of the Hepaticæ. There seems no question that the so-called mother cell of the archegonium in all the ferns is really homologous only with the axial row of cells of the bryophyte archegonium, the four rows of neck cells being a further development of the terminal cap cell of the liverwort archegonium.

The inner of the two primary cells, as we have already stated, may have a basal cell cut off from it before the further divisions arise, by which the egg cell and the canal cells are divided. The neck canal cell is very broad and may become divided into two cells, but usually the division is confined to the nucleus, which probably divides in all cases. The ventral canal cell cut off in the usual fashion from the egg is, with the exception of *Danæa*, very large and conspicuous, thus differing from
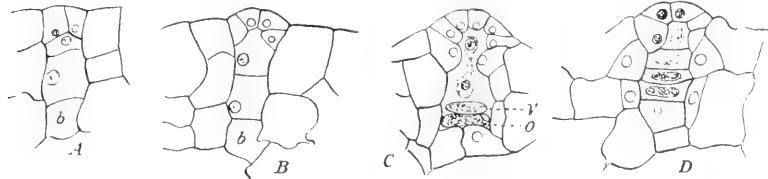


FIG. 101.

Archegonia of *Angiopteris*. ×275. *b*, basal cell; *o*, egg; *v*, ventral canal cell.

*Ophioglossum*, where the ventral canal cell is so difficult to demonstrate; but in *Danæa* there is the same imperfect development of the ventral canal cell that is found in *Ophioglossum*.

As the archegonium approaches maturity a layer of mantle cells, much like those which surround the antheridium, is cut off from the tissue surrounding the venter of the archegonium. The archegonia of *Marattia douglasii* are confined to the lower side of the midrib and begin to form at some distance back of the growing point; so far as can be determined, any superficial cell of the apical meristem can develop into an archegonium. The mother cell divides, as we have seen, into three superimposed cells, of which the lowest, *b*, usually divides later by vertical walls, and forms the base of the archegonium. From the central one, by transverse divisions, are formed the canal cells and egg, and from the uppermost the neck. Compared with the typical ferns, the most striking differences are the short neck and the very broad canal cells. The cover cell undergoes division into four, by two intersecting vertical walls, as in *Ophioglossum*, and each of these four cells then undergoes division by nearly horizontal walls, but the cells remain short, so that the neck projects very little and there are only three or four cells in each row; occasionally there may be only two. Jonkmann states that, as a rule, two of the rows of the neck contain three cells and two contain four, but that there may occasionally be as many as five. The neck canal cell often shows a trace of a division and there may be an actual division wall formed (fig. 101, *D*), but in *Marattia douglasii*

this is not ordinarily the case. In one instance in this species I observed a division by a vertical wall, so that two neck canal cells were formed, placed side by side, in a way recalling very strongly the division of the neck canal cell described by Jeffrey in certain species of *Equisetum* (Jeffrey 2). The central cell is divided again by a horizontal wall into two nearly equal cells, the lower one being the egg, the upper one the ventral canal cell. The mature egg is nearly elliptical in form, the upper third being almost homogeneous and quite colorless, forming the so-called receptive spot. The nucleus is of moderate size and does not stain very strongly. The archegonium of *Angiopteris* closely resembles that of *Marattia*, but is perhaps somewhat narrower (fig. 101).

Jonkmann figures the archegonium of *M. cicutæfolia*, which shows that this species also is, in the relative size of the canal and neck cells, very much like *Angiopteris*. Farmer thought that a basal cell was usually, if not always, present, and my own studies tend to confirm this.
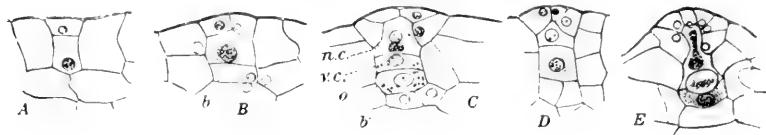


Fig. 102.

Archegonia of *Kaulfussia*. ×210. *b*, basal cell; *o*, egg; *v*, ventral canal cell; *n.c.*, neck canal cell.

In *Kaulfussia* (fig. 102) the archegonia form, as a rule, only after the antheridia have ceased to develop. Compared with the other Marattiaceæ they are decidedly large, and in this respect *Kaulfussia* approaches *Ophioglossum*. Like the mother cell of the antheridium, there is a good deal of variation in the width of the young archegonium in *Kaulfussia*. Some of the narrower types recall the archegonium of *Anthoceros* and emphasize the resemblances between the archegonium of the Anthocerotaceæ and the eusporangiate ferns. The neck of the archegonium is very short, each of the four original neck cells often dividing only once, so that there may be but two cells to each of the four rows. More commonly, however, there is a
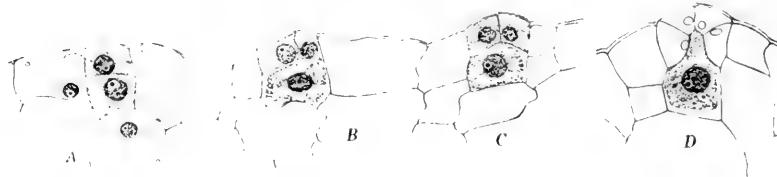


Fig. 103.

Young archegonia of *Danæa elliptica*. ×350.

second division in some of the cells, so that each row consists of three cells. The nucleus of the broad neck canal cell probably always divides, but the number of available specimens was too small to decide whether or not there is ever formed a division wall between these, although it is not at all unlikely that this may occur. The ventral canal cell is conspicuous and equals its sister cell, the egg, in breadth. In the peripheral portion of all the axial cells of the archegonium there are many small granules of starch. Jonkmann figures similar starch granules in both *Marattia* and *Angiopteris*.

The archegonium in *Danæa*, while resembling that of the other Marattiaceæ, in its position and early development shows some marked differences, the significance of which is not quite clear. The division of the mother cell into the primary neck cell and the central cell follows in the same way as in the other forms, but the inner cell, usually at least, does not have a basal cell separated from it, but develops at once into the egg cell and canal cells. The absence of the basal cell is by no means unknown, however, among the other Marattiaceæ. The primary neck cell gives rise to the usual four cells, each of which divides into three or four, and exceptionally into five. There is a marked elongation of the inner cell before its separation into the central cell and the neck canal cell (fig. 103, *D*).
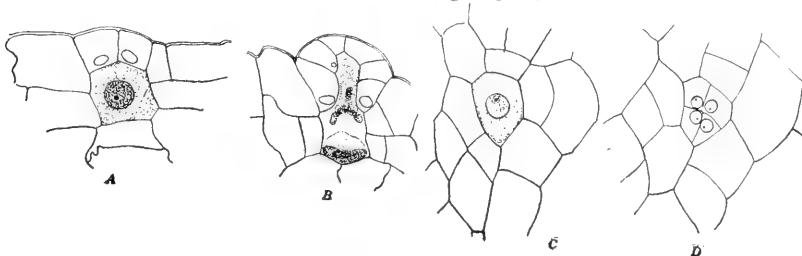


Fig. 104.

A, young, B, nearly mature, archegonium of *D. jamaicensis*.  ×360.
C, D, cross-section of a young archegonium of same species. D shows the four primary neck cells.

Up to this point there is nothing peculiar in the development of the archegonium in *Danæa*, but while in most of the other Marattiaceæ a conspicuous ventral canal cell is formed, its sister cell being the egg, in *Danæa* the formation of a definite ventral canal cell could not be satisfactorily demonstrated. In a number of cases (fig. 105; plate 2, fig. 45) a small nucleus-like body could be seen in a large, clear
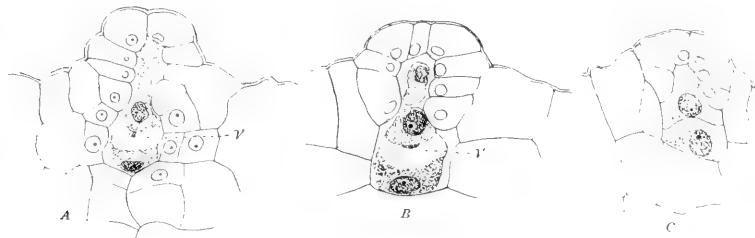


Fig. 105.

Three mature archegonia of *Danæa elliptica*.  ×350.
A and B show traces of a ventral canal cell; *v*, in B, a division of the neck canal cell.

space just above the somewhat contracted mass of protoplasm, with its large and conspicuous nucleus, which constitutes the egg cell; but this ventral canal cell nucleus, if such it is, is very different in appearance from the large and conspicuous one found in the other Marattiaceæ, and in the absence of any division stages its nuclear nature must for the present remain somewhat doubtful.

There is somewhat the same uncertainty in regard to the primary division of the neck canal cell. This possesses a large and conspicuous nucleus which in large and apparently mature archegonia was still undivided. In a few cases a division of the protoplasm in the neck cells was observed; in other cases, without any such division,

a second body of rather indefinite outline and staining much less strongly than the lower nucleus could be made out; but up to the present time I have not been able to satisfy myself that the division of the neck canal cell nucleus, which always occurs in the other Marattiaceæ, takes place here. The very uncertain nature of the ventral canal cell recalls strongly the condition of affairs in *Ophioglossum*, where it is equally difficult to demonstrate satisfactorily the presence of a true ventral canal cell.

## FERTILIZATION.

It has not been possible to follow in detail the process of fertilization in the Marattiaceæ, but several stages were found in preparations of *Marattia douglasii*. The entrance of the spermatozoid into the archegonium was not seen, but in a number of cases the material had been killed immediately after, and twice spermatozoids were found which had penetrated into the egg. In these cases the spermatozoid was quite unchanged in form, but had not yet entered the nucleus itself. One case was observed where there were apparently two nuclei in close contact, but the egg nucleus was much contracted and it is doubtful whether this was really a normal appearance. It is probable that the details of fertilization are quite similar to those observed in other ferns.

## II. THE EMBRYO.

The study of the embryogeny of the Marattiaceæ offers many difficulties. Fertilization does not seem to be of common occurrence and a very large number of prothallia must be examined before even a small series of embryos can be secured; moreover, the earlier stages of the embryo are peculiarly liable to shrinkage in preparing them for sectioning and it is exceedingly difficult to secure really satisfactory preparations of these early stages.

A marked peculiarity of the young sporophyte, which was first shown by Luerssen and Jonkmann for *Marattia* and *Angiopteris*, is the orientation of the primary organs of the young sporophyte with reference to the archegonium. The primary or basal wall in the embryo is always transverse, as it usually is in *Ophioglossum*, instead of being vertical as in most of the typical ferns. The first leaf, instead of being formed from the portion of the embryo nearest the archegonium, as it is in the common ferns, arises from the half of the embryo which is turned away from the archegonium, and grows straight upward, bursting through the prothallium upon its upper surface, instead of appearing upon the lower side of the prothallium and curving upward. The external organs of the young sporophyte are differentiated ✕
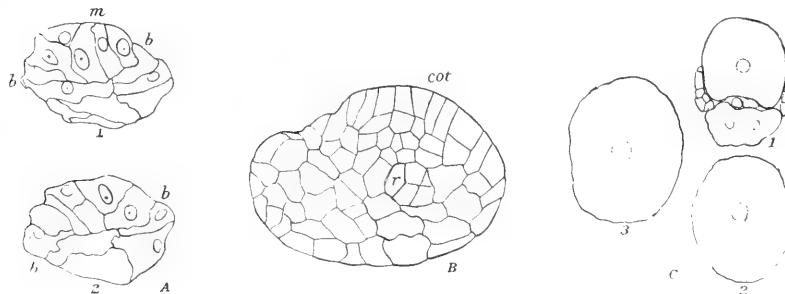


FIG. 106.—*Marattia douglasii.*

A. Two longitudinal sections of a young embryo. *b b*, basal wall. ✕200.
B. A similar section of an older embryo. *cot*, cotyledon; *r*, root initial.
C. Three transverse sections of a much older embryo, showing junction of the two first leaf traces. ✕50.

much later than is the case in the Leptosporangiates, and the Marattiaceæ in this respect closely approach the Ophioglossaceæ.

It has been generally assumed that, as in the leptosporangiate ferns, the cotyledon and stem are of epibasal origin, the root and foot hypobasal. This conclusion was reached by both Jonkmann and Farmer, and my early studies on *Marattia douglasii* led me to the same conclusion. A further study of this species, however, as well as an examination of the embryos of *Angiopteris*, *Kaulfussia*, and *Danæa*, has shown that this is not the case, but that the whole of the hypobasal region is devoted to the formation of the foot, and the root is developed secondarily from the epibasal region, from which are also derived the stem apex and the cotyledon.

While in the typical ferns the young organs of the embryo at a very early stage show a definite apical growth, the apical cells being readily traceable to the primary octants of the embryo, this is by no means so readily shown in the Marattiaceæ. In the later stages, such initial cells can be seen in the root and stem at least, but the relation of these initial cells of the root and stem to the early divisions of the embryo is very difficult to determine. In *M. douglasii* (Campbell **3**) I stated that there

was probably a single initial in the stem and in the primary root, and Farmer also thought that a single root initial was always present in *Angiopteris*, but concluded that such an initial cell was not present in the stem of the young sporophyte. Brebner (Brebner 2) says that in *Danæa simplicifolia* such a single initial cell seemed to be always present in the stem, and my studies on *Angiopteris*, *Kaulfussia*, and *Danæa* indicate that a single initial is developed at an early period in the stem apex and persists until the sporophyte has developed several leaves.

Before any division occurs in the embryo the fertilized cell increases markedly in size, after which there is formed the horizontal basal wall (figs. 106, 109, *b b*). This is probably followed at once by the median wall (except in *Danæa*), so that the embryo at this stage is divided into four approximately equal quadrants (see Jonkmann 3, fig. 9).

The genus *Danæa*, at least this is true for *D. jamaicensis* and *D. elliptica*, differs in the early divisions of the embryo from the other Marattiaceæ. The egg cell after fertilization elongates in a way which closely resembles that found in *Botrychium obliquum* (Lyon 1). The primary hypobasal cell either divides no further or only once and forms a short suspensor, so that all the organs of the young embryo, including the foot, are really of epibasal origin. As yet no trace of such a suspensor has been found in the other Marattiaceæ.

### THE EMBRYO OF MARATTIA.

Luerssen (Luerssen 2) found the one-celled embryo and young plants of *Marattia cicutæfolia*, but was not able to procure the older embryos. The writer (Campbell 3) succeeded in procuring several stages of the embryo in *Marattia douglasii*, and somewhat later Luerssen described and figured some of the earlier stages in *M. fraxinea* and *M. weinmanniæfolia*. These, so far as I am aware, complete the list of records upon the embryo of *Marattia*.

The fertilized ovum in *Marattia douglasii* becomes much enlarged before it divides and completely fills the venter of the archegonium. The granular contents of the egg cell become evenly distributed without any apparent increase in quantity as the fertilized ovum grows, so that the one-cell embryo contains comparatively little granular contents, but the nucleus is very conspicuous. At the time of the first division the young embryo is almost perfectly globular in form. I was unable to find the stages immediately following this, but Jonkmann has figured an eight-cell embryo of *M. fraxinea* (see Jonkmann 3, fig. 9). The basal wall is transverse and this is followed successively by the median and transverse walls, so that the globular embryo is divided into approximately equal octants. From the hypobasal half, which is nearest the archegonium, there is developed the foot alone, while all of the other organs arise from the epibasal portion, which is turned away from the archegonium.

The youngest embryo (except the one-celled stage) which I found in *M. douglasii*, is shown in fig. 106, *A*. This is about the same stage as the one shown in Jonkmann's figs. 11 and 13. Unfortunately this embryo was rather badly shrunken in the process of embedding and the division walls were a good deal distorted, so that it is rather hard to determine exactly their correct relation to each other; but probably the wall *b b* represents the basal wall and *m* the median wall. The embryo has now lost its original globular form and become oval, the long axis lying transversely. The secondary divisions in the octants are mostly anticlinals, and the first periclinals have just appeared in a few of the cells. Figure 106, *A*, shows two consecutive median sections. It will be seen that cell division is more active in the epibasal portion than in the hypobasal region.

In my original study of *Marattia* I concluded that the primary divisions resulted in the establishment of the organs of the young sporophyte in a manner similar to that in the Leptosporangiatæ, i. e., that the stem and leaf were derived from the epibasal quadrant, the root and foot from the hypobasal ones. Farmer thought that this was true for *Angiopteris* and Jonkmann assumes that it is the case also in *Marattia*. A further study of my preparations, however, has led me to believe that the whole of the hypobasal region is devoted to the foot, while the root, together with the leaf and stem apex, are of epibasal origin. A large cell (fig. 106, *A*) occupies approximately the same position as the stem apex in the older embryo and it is possible that this may be the apical cell of the stem, but in the absence of the intermediate stages this can not be positively asserted. For a long time the embryo retains the oval form and there is scarcely any sign of the young organs of the sporophyte which in the leptosporangiate ferns are so early manifest; indeed, Jonkmann states that there is no differentiation at all, but a careful study of the older embryo shows unmistakable indications of the definitive organs. The development of the tissue in the epibasal region is not uniform, but somewhat to one side of the center (fig. 106, *B*) there may be seen a group of columnar superficial cells, which mark the position of the future growing point of the stem. Whether one of these superficial cells is the definitive apical cell it is impossible to determine, but from a comparison with the older stages, as well as with corresponding stages of the embryo in the other genera, it seems probable, at least, that this is the case, and perhaps this initial cell may be traced back to the centrally placed cell noted in the younger embryo. A considerable portion of the epibasal tissue is not included in the meristematic region, which is derived mainly, at least, from only one of the original epibasal quadrants. The



FIG. 107.

Two cross-sections of an old embryo of *Marattia douglasii*. A passes through the stem apex, *st*, and base of cotyledon. B passes through root apex, *r*. ×200.

tissue adjacent to this meristematic region is made up of large cells with less dense contents and smaller nuclei and differs in no way from the large cells of the foot, into which this tissue insensibly merges and of which it may be said to constitute a part.

In the embryo figured, it was not quite certain whether the root apex had begun to form or not. It seems probable that the cell *r* is really the apical cell of the young root, but except for its position it was not noticeably different from the cells adjacent; however, as in *Angiopteris* and *Danæa* there is no question that the root originates in this position, it is probable that this cell is really the initial for the young root and is cut out from the base of the epibasal tissue near its junction with the foot. This marked endogenous formation of the root is very much like that in the embryo of *Ophioglossum moluccanum*, but differs entirely from the superficial origin of the apical cell of the root in the embryo of the Leptosporangiates.

The stem apex in the older embryo is of very limited extent, consisting of only a few cells, of which one, as we have said, is probably the definite apical cell, although it must be said that this point is very difficult to decide, as all of the central cells of this apical group look a good deal alike, but a careful study of both transverse and longitudinal sections seems to point to one of these as the apical cell, which in shape is a good deal like that in the stem apex of *Ophioglossum*. In longitudinal

sections this is somewhat wedge-shaped, the narrow end turned outward and the broad base below. In cross-section this cell is nearly square in shape (fig. 107, $A$, $x$) and there may be seen a fairly regular series of four segments cut from the lateral faces. From the broad truncate base of the cell, segments are also cut off which contribute to the inner tissue of the stem.[*]

Much the greater part of the epibasal meristem contributes to the cotyledon, which is soon evident as a broadly conical protuberance, somewhat flattened on the side adjacent to the stem apex and merging gradually on the outer side into the large-celled tissue which adjoins the foot. To judge from the limited number of young embryos which I could examine, it seems that the growth of the cotyledon is not due to the activity of a single apical cell, but this point was somewhat uncertain.

By the time that the cotyledon is established, growth has progressed in the young root, which now is seen to have a conspicuous apical cell, which divides rapidly so that the root quickly elongates in a direction opposite to that of the cotyledon (fig. 108). The apical cell of the primary root in *M. douglasii* is not triangular in outline, but more or less quadrilateral, whether seen in longitudinal or cross sections (fig. 107, $B$). In form and segmentation it perhaps more nearly resembles that of *Danæa* than it does the tetrahedral apical cell which occurs in the young primary root of *Angiopteris*.



Fig. 108.

A. Section of an advanced embryo of *Marattia douglasii*, just before emergence of cotyledon.
B. The cotyledon, more enlarged.

Active cell divisions take place also in the tissue of the foot, which completely incloses the young root and becomes practically merged with it, so that it is quite impossible to say, at the time the root emerges, just how much of the tissue of its outer portion really belongs to the root itself and how much is derived from the original tissue of the foot. The latter is now no longer recognizable as such, the young sporophyte apparently at this time being composed almost entirely of the cotyledon and the very large root, with the stem apex, lying near their junction. From a comparison with younger stages, however, it is perfectly evident that the enlarged central region of the embryo at this time is composed mainly of tissue belonging to the foot, which is, so to speak, perforated by the root in its downward growth.

The development of the vascular bundles at a very early period is first evident in the cotyledon, which almost as soon as it can be recognized at all is seen to have a strand of procambium extending below it. If this procambium strand is traced downward, it is seen to continue without interruption into the base of the root, exactly as it does in *Ophioglossum moluccanum*. No trace of a procambial cylinder can be found extending into the stem apex, which gives rise only to the parenchyma of the central pith. This early development of the vascular bundles in the cotyledon and root was correctly observed by Jonkmann and Farmer, both for *Marattia* and

[*] In a very recent paper in the Botanical Gazette (Feb. 1911), Miss Charles states that the apical cell of the stem in *Marattia alata* is at first triangular in cross-section. In older sporophytes the single apical cell was replaced by a group of initial cells.

*Angiopteris*, but they seem to take it for granted that a vascular strand is also developed in the stem; at any rate they make no reference to the absence of such a vascular strand from the stem region. In my earlier study of *Marattia*, I supposed that a strand was developed which belonged to the stem itself, but a further examination of many plants, after a study of this point in the other genera, has made it pretty clear that this supposed stem bundle really belongs to the second leaf, the rudiment of which appears at a very early period.

### THE EMBRYO OF ANGIOPTERIS.

In *Angiopteris* the embryo very early becomes more greatly elongated transversely than is the case in *Marattia*, so that in a longitudinal section it appears as a very much depressed oval (fig. 109); the epibasal region is larger than the hypobasal
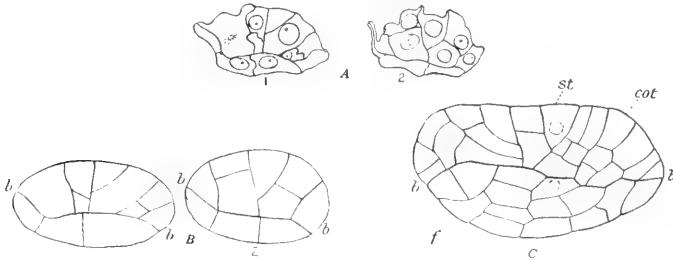


FIG. 109.

A. Two sections of a young embryo of *Angiopteris*. The cells were badly shrunken.
B. Diagrams showing arrangement of cells of same embryo.
C. An older embryo. *b b*, basal wall; *st*, stem apex; *cot*, cotyledon; *f*, foot.

and the quadrant divisions are often still evident. Whether octant divisions are formed in all the quadrants could not positively be determined, but Jonkmann states that such is the case. There can usually be found in the young embryo a nearly centrally placed large cell (fig. 109, *C*, *st*), which probably is the initial cell of the young stem. The position of this cell is not unlike that which occurs in the
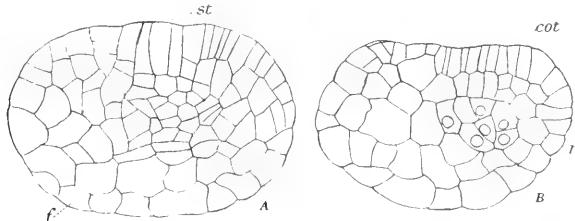


FIG. 110.

Two longitudinal sections of an older embryo of *Angiopteris*. ×200. The young root, *r*, is shown in *B*. *st*, stem apex; *cot*, cotyledon; *f*, foot.

embryo of *Equisetum*, and at this stage there is also a certain resemblance to the embryo of *Botrychium virginianum*. As the embryo grows it tends to assume a more nearly globular form (fig. 110). The basal wall can still be imperfectly followed, the hypobasal portion of the embryo being made up of the large cells forming the foot, while above the basal wall the cell divisions are more active and the rudiments of

the stem and cotyledon can be recognized, although the embryo still retains its oval outline. As in the embryo of *Marattia*, growth is most active in the central region of the epibasal part of the embryo and there can generally be recognized a large central cell, which is presumably the single initial of the stem apex, although, as in the case of *Marattia*, it is not absolutely certain that a single initial cell is always present. Cell division is especially active on one side of the stem apex, and this area marks the position of the young cotyledon. The limits of this growth area are
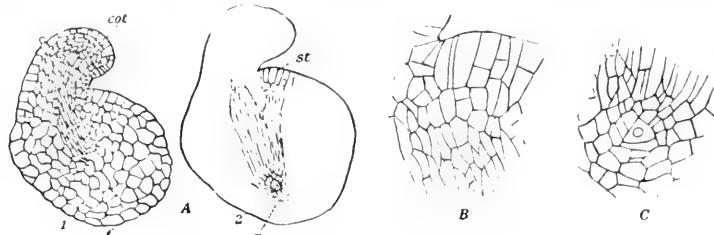


Fig. 111

A. Two longitudinal sections of an old embryo of *Angiopteris*. ×75
B. Stem region of same embryo. ×160.
C. Root apex. ×160.

not very sharply defined and it is difficult to say whether it can be traced back to a single quadrant or octant cell, but it is probably not always constant in its position; indeed, the stem looks as if the stem apex and the cotyledon both arose from the same quadrant, the second epibasal quadrant contributing, at least in part, to the foot.

As seems to be true in all the Marattiaceæ, the root makes its appearance at a comparatively late period and it is evident that the root, as in the embryo of *Marattia*, is a strictly endogenous structure. The first indication of the root is the development
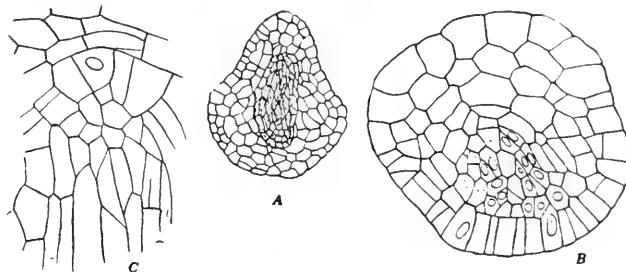


Fig. 112.

A. Longitudinal section of an advanced embryo of *Angiopteris*, cut in the plane of the lamina of the cotyledon. ×50.
B. Cotyledon of same embryo, showing dichotomy of apex. ×220.
C. Root apex of same. ×220.

of a group of actively dividing cells, almost in the center of the embryo below the stem apex. This meristematic region is probably always of epibasal origin, but it is close to the basal wall and it is possible that sometimes it may arise below it. The apical cell, which in the early stages almost always appears triangular in section, becomes conspicuous and was recognized by Farmer in his study of the embryo of

*Angiopteris* (Farmer 1). The root initial now divides by regular segmentation and the root apex pushes rapidly down through the underlying foot and ultimately emerges on the lower side of the prothallium. In the meantime the cotyledon grows actively and there is a rapid elongation of the whole embryo in a vertical direction. As in the case of *Marattia*, it is difficult to prove that the cotyledon grows from a single initial cell. The cotyledon has very much the form of that in *Marattia*, growth being more active on the outer side, so that it curves over the stem apex very much as the cotyledon does in the embryo of *Botrychium virginianum*. About the time that the cotyledon is ready to emerge, the apex becomes flattened out and (sometimes, at any rate) there is a true dichotomy of this apex (see fig. 112, *B*).

In the primary root there seems to be no question of the presence of the single initial cell which, at first at least, has the tetrahedral form, but later on is apt to have the base truncate, although it usually has three series of lateral segments. I have not been able to confirm Farmer's statements that the single apical cell is later replaced by a group of similar initials, as in the later roots of the sporophyte, although it is not at all impossible that such may sometimes be the case. The development of the embryo of the vascular bundles in the young sporophyte is exactly the same as in the corresponding stages of the embryo in *Marattia*.

### THE EMBRYO OF KAULFUSSIA.

So far as I am aware, no account has been published of the embryo in *Kaulfussia*, except one of my own (Campbell 11). Only two young embryos were found, so that it was impossible to follow in detail the early history of the young sporophyte. The basal wall, as in the other Marattiaceæ, is transverse and, to judge from a comparison of similar stages of the embryo in *Marattia* and *Angiopteris*, all of the organs of the young sporophyte in *Kaulfussia*, except the foot, are also of epibasal origin. Figure 113, *A*, shows a nearly median longitudinal section of the young embryo. This is very much elongated transversely, and to judge from the position of the cells the basal wall is probably followed by the median walls, forming nearly equal quadrants. The large cell (*st*) in one of the epibasal quadrants corresponds in position to the similar cell found in the embryos of the other genera and very likely may represent the primary initial cell of the stem. The embryos, however,
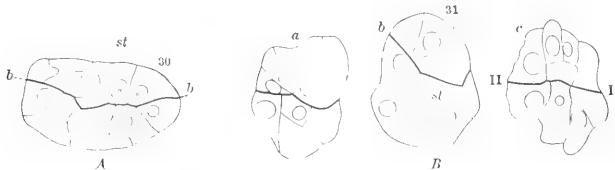


FIG. 113. —Young embryos of *Kaulfussia*. ×275.

A. Longitudinal section.
B. Three cross-sections of a similar embryo. *b b*, basal wall; II, quadrant wall.

were too young to make clear the relation of the cotyledon and primary root to the stem. Three nearly transverse sections of an embryo of about the same age are shown in fig. 113, *B*. To judge from the structure of the older sporophyte at the time when it first emerges from the prothallium, *Kaulfussia* agrees in the main with *Marattia* and *Angiopteris* in the origin of the young organs of the sporophyte.

## THE EMBRYO OF DANÆA.

Brebner (Brebner 2) has described the older embryo of *Danæa simplicifolia*, but did not secure the earlier stages. The youngest specimens he figures closely resemble in form a corresponding stage in the species studied by me, and the slightly pointed basal region suggests the possibility of the presence of a short suspensor in *D. simplicifolia* like that which I have found in *D. jamaicensis* and *D. elliptica*. While my own collection of young embryos is not as complete as might be wished, still enough stages were secured to show that at least *D. jamaicensis* and *D. elliptica*
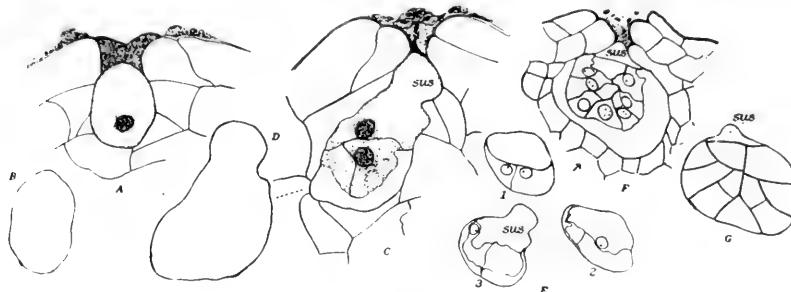


FIG. 114. -*Danæa jamaicensis.*

A. Archegonium containing a one-celled embryo.
B. Outline of next section of same embryo.
C. Three-celled embryo, showing suspensor, *sus*.
D. Outline of next section of the same embryo.

E. Three longitudinal sections of a four-celled embryo.
F. An older embryo, which was shrunken.
G. Diagram of F, showing probable arrangement of cells.

differ remarkably from the other Marattiaceæ that have been studied in the development of a short suspensor, thus showing an interesting analogy with the embryo of *Botrychium obliquum*, described by Lyon (Lyon 1). Whether *D. jenmani* shows the same peculiarity was not determined, on account of the failure to obtain the young embryo of this species; but as in its later development it corresponds very closely to the other species, it is highly probable that a suspensor is developed.



FIG. 115
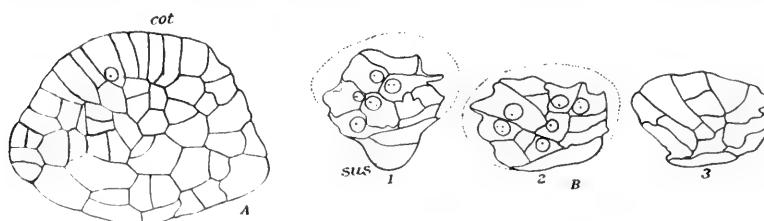
A. A nearly median section of an older embryo of *D. jamaicensis*. The suspensor does not show in this section.
B. Three sections of a young embryo of *D. elliptica. sus*, suspensor.    × 200.

Before the first division takes place in the embryo, the fertilized ovum enlarges to several times its original size and becomes decidedly elongated. The first division wall, as in the other Marattiaceæ, is transverse, but of the two primary cells thus formed the hypobasal one divides no further, or only once, and forms a short suspensor which pushes up into the neck of the archegonium whose cells become more or less completely disorganized (fig. 114, *A*). The next division wall is a nearly median one in the epibasal cell, and this is soon followed by a second wall in each

of the epibasal cells, so that this portion of the embryo is divided into four nearly equal quadrants. There is some evidence that these are followed by horizontal octant walls, so that the epibasal region is thus divided into octants in much the same fashion as obtains in the whole embryo in other Marattiaceæ and in the leptosporangiate ferns. How far these divisions are constant can only be conjectured, owing to the small number of young embryos which were available. The elongated pear-shaped embryo in *Danæa* appears very different indeed, in these early stages, from the broadly elliptical and much depressed embryos of corresponding stages in the other Marattiaceæ (fig. 114, *C, D, E*).
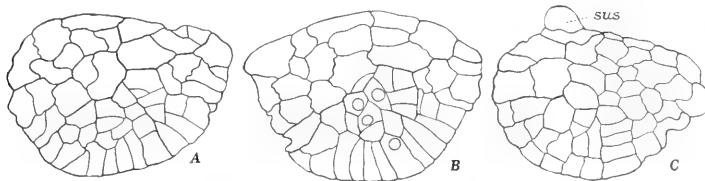


FIG. 116.—*D. jamaicensis.*

Three sections of an older embryo. ×200. Section B is a nearly median section; A is the next section in the series; C shows the suspensor, *sus*.

A further study of the embryos shows that all of the lower half of the epibasal region, probably that derived from the four lower octants—i. e., the four octants that were in contact with the suspensor—develops into the foot, while all of the other organs of the embryo (leaf, stem, and root) arise from the four terminal octants.

Quite early in the development of the embryo, there appears the same centrally placed large cell which we have observed in the embryos of the other genera and which, as we have seen, probably represents the primary stem initial. Brebner concluded that a single initial was present in the stem of *D. simplicifolia* and this is true of the three species examined by me. The initial of the stem in *Danæa* becomes
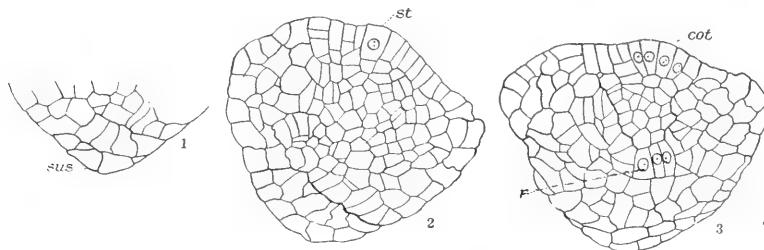


FIG. 117.—*D. elliptica.*

Three sections of a large embryo. ×150. *sus*, suspensor; *st*, stem apex; *cot*, cotyledon; *r*, root initial.

very easily seen in the older stages and is perhaps more clearly defined than it is in any other of the Marattiaceæ. Whether or not this cell can be traced-back to one of the original terminal octant cells is difficult to say, but it is quite possible.

No single initial could be made out for the cotyledon, and it seems quite likely that the position of the cotyledon is not always exactly the same, being determined perhaps by the position of the embryo with reference to the light or to some other factor. The cotyledon, as in *Marattia* and *Angiopteris*, first appears as a slight prominence close to the nearly centrally placed stem apex, and there is soon visible

a group of actively dividing cells which constitute its growing point, but no one of these can be certainly named as the apical cell (fig. 117, 3). The embryo now rapidly increases in breadth until the apex is almost flat, except for the slight projection in the center, where are situated the stem apex and the young cotyledon. The whole embryo at this stage may be described as "top-shaped." The stem and cotyledon occupy a comparatively small portion of this broad terminal area and are surrounded by a ring of large absorbent cells, physiologically, at least, belonging to the foot and merging insensibly into the similar cells which make up the lower half of the embryo.
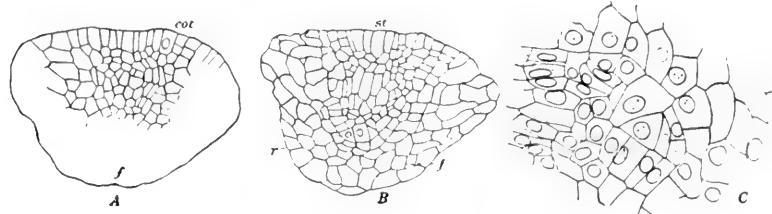


FIG. 118.—*D. jamaicensis.*

A, B. Two sections of a large embryo. ×150. *cot*, cotyledon; *st*, stem; *f*, foot.   C. Root apex of same embryo.   ×360.

As in the other Marattiaceæ, the very young embryo shows no trace at all of the root and this appears only after the embryo has reached a comparatively large size. The origin of the root is exactly the same as in the other genera and it is a strictly endogenous structure. The single initial cell arises deep down in the tissue of the embryo, usually below the cotyledon and probably from the same octant as that from which the cotyledon is formed, but owing to the displacement of the original division walls this can not be determined positively and it may be that the position of the root is not always exactly the same. The apical cell of the root soon
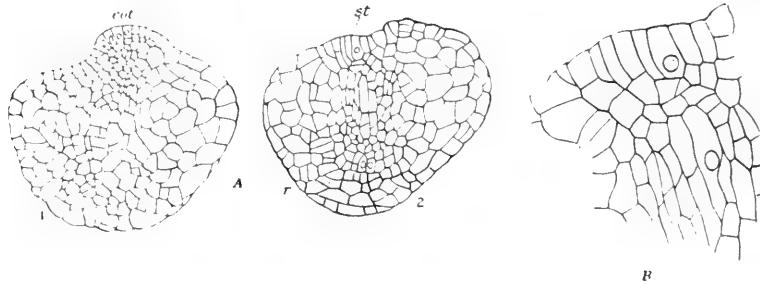


FIG. 119.—*D. jamaicensis.*

A. Two sections of an advanced embryo.   ×100.   B. Stem apex of the same embryo.   ×220.

becomes conspicuous, but it does not usually show the triangular form as seen in a longitudinal section, but appears more nearly square, as in *Marattia.* This root initial cell is quite variable in form, but more commonly it appears in longitudinal section with a truncate base (fig. 117, *r*). In transverse section it approaches the triangular form, but is more or less irregular in outline. The lateral segments cut off from the initial cell are large and contribute later to the root cap as well as to the inner root tissues, and it is not impossible (as Farmer believes to be the case also

in *Angiopteris*) that sometimes more than one of these terminal cells function as the apical cell.

As soon as the root apex is established its growth is very rapid and, as in the other genera, there is a rapid elongation of the whole embryo, whose vertical diameter very soon becomes greater than the transverse diameter, and ultimately the whole embryo becomes very much elongated. In the axial region of the embryo just below the stem apex there is a marked elongation of the central cells which might at first sight be taken to represent the central vascular cylinder, but these elongated cells do not give rise to vascular elements, but remain as elongated parenchyma and belong really to the pith. The part of the foot which lies below the growing root apex acts as a very massive root cap and is pushed down with the growth of the root until the latter emerges from the lower side of the prothallium. The cotyledon in the meantime grows actively upward and finally penetrates the prothallium, emerging
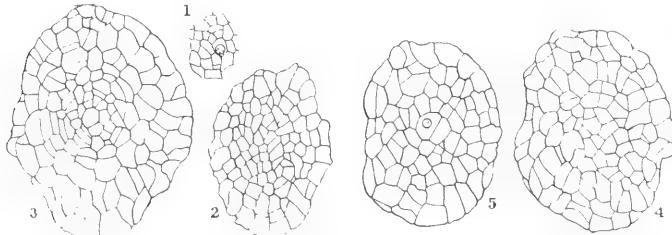


FIG. 120.—*D. jamaicensis.*

Five of a series of transverse sections from an advanced embryo. ×200. 1 passes through stem apex; 5 shows apex of root.

on the upper side. The growth of the stem is slight and the cotyledon and root form nearly a straight line, as in the other Marattiaceæ and in *Ophioglossum*, so that the young sporophyte may be described as bipolar. The equatorial region is surrounded by the large absorbent cells in contact with the prothallium and all of these may be said to function as the foot, although it is impossible to say how much of this tissue is derived from the original foot.

### THE ANATOMY AND HISTOLOGY OF THE YOUNG SPOROPHYTE.

The young sporophyte, at the time the root emerges from the prothallium, is very much alike in all of the genera (figs. 108, 111, 121). The cotyledon at this stage is a thick, conical protuberance, strongly curved inward over the stem apex, owing to the more rapid growth upon its outer side. Close to its base lies the very limited growing region of the stem, adjacent to which is the rudiment of the second leaf, which at this time projects very slightly above the level of the stem apex. The cells of the latter, and also those of the young leaves, are evidently actively growing cells with conspicuous nuclei and are quite different in appearance from the large, transparent cells that compose the enlarged mid-region of the young sporophyte. This is largely made up of the original tissue of the foot, but this tissue merges insensibly into the basal region of the cotyledon and the upper part of the root, these two organs forming in their growth almost a straight line. Whether or not we term this middle region of the young plant the "stem," it must be borne in mind that it does not arise from the activity of the extremely limited meristem, forming the true stem apex. The young sporophyte is traversed by a single conspicuous vascular bundle which extends through the cotyledon and root without inter-

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ruption, and it is quite impossible to say just exactly where the point of junction is. In *Angiopteris* elongated tannin cells, which stain very strongly, accompany the vascular bundle, both in the root and cotyledon; but these are either entirely wanting or but slightly developed in *Marattia* and are quite absent from the young sporophyte at this stage in the other genera. In the relation of the primary root and leaf, therefore, the embryo of the young sporophyte in the Marattiaceæ shows a most striking resemblance to the condition found in *Ophioglossum moluccanum*. The cotyledon, as in *Ophioglossum*, is not to be looked upon as an appendage of the stem, but as an organ *sui generis*.

Almost as soon as the second leaf is recognizable, there is evident, connecting it with the primary vascular strand, a short group of procambium cells, and the stem apex is seen to occupy the space between these two leaf traces. *No procambium is developed in the stem above the junction of the leaf traces, but the inner cells, derived from the apical meristem, contribute solely to the medullary tissue of the sporophyte* (fig. 121, *A*).
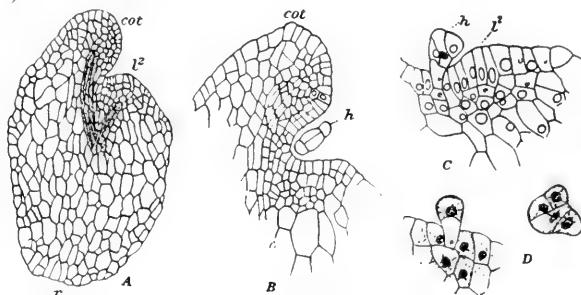


FIG. 121.—*Danæa jamaicensis*.

A. Large embryo. *cot*, cotyledon; *l²*, second leaf. ×50.  C. Second leaf. ×150.
B. Median section of cotyledon; *h*, epidermal scale. ×95.  D. Trichomes from apical region.

The first tracheary tissue arises in the mid-region of the young sporophyte and consists of a group of short reticulate tracheids. From this point the development of the tracheary tissue proceeds upward into the cotyledon and downward into the root. The single strand of tracheary tissue in the cotyledon is seen to be continuous with one of the xylem masses of the diarch root. The second xylem of the root arises somewhat later and is connected with the xylem of the second leaf. This second xylem mass is decidedly smaller than the first one, at least in *Danæa*, where I have studied this point carefully, and an examination of the other genera points to a similar inequality in the xylems of these as well, but this may not always be the case.

### THE COTYLEDON.

The cotyledon at a very early period bends strongly over the stem apex, very much as it does in *Botrychium virginianum*, and very soon afterwards begins to flatten out, so as to indicate the separation of the lamina from the petiole. The flattening of the apex is followed, in many cases at least, by a true dichotomy, which is soon repeated, so that the lamina becomes fan-shaped, with a strictly dichotomous venation (fig. 87, *G, H*; fig. 127). This shows especially well in *Marattia douglasii*. In *M. sambucina* (fig. 87, *C*) the cotyledon is more nearly orbicular than in *M. douglasii*, but the venation indicates a similar early dichotomy of the apex.

In *Angiopteris*, the form of the cotyledon is extremely variable (fig. 124). Farmer states that it has a distinct midrib extending to the apex of the cotyledon

and that the secondary veins are pinnately arranged with reference to the midrib. These later veins, however, may show dichotomous branching. I made an examination of a considerable number of young sporophytes collected at the same place where Professor Farmer secured his specimens, but very few of the plants that I collected showed this pinnate venation in the cotyledon, although it is usually conspicuous



FIG. 122.

Young sporophyte of *Marattia douglasii* still attached to the gametophyte, *pr*; Natural size.
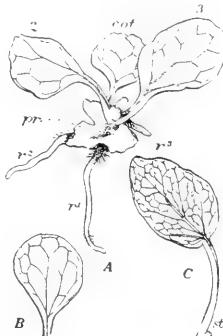
FIG. 123.

A. Young sporophyte of *Kaulfussia* attached to gametophyte, *pr*. ×2.
B. Cotyledon from another plant. ×2.
C. A later leaf; *st*, stipule. ×1.

in the second leaf. Figure 124, *C*, shows drawings of a number of the forms from my collection, showing the variation from a strictly pinnate to a perfectly dichotomous venation. Many of them show an intermediate venation, but with very few exceptions, even where a midrib was present, this was forked at the apex, indicating
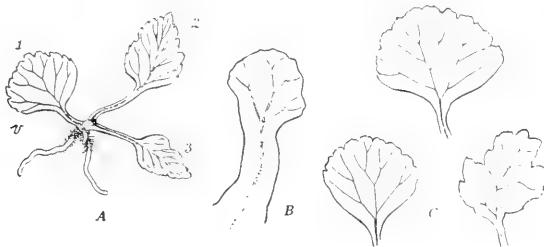


FIG. 124.

A. Young sporophyte of *Angiopteris*, with three leaves. 1, the cotyledon. ×2.
B. Young cotyledon. ×20.
C. Three cotyledons, showing the variation in form. ×3.

that there had been an early dichotomy. This primary dichotomy of the apex was well shown in a section through a very young leaf (fig. 112, *B*) where there was no question that such a dichotomy was taking place.

In *Danæa* there is much the same variation in the form of the cotyledon as in *Angiopteris*. Brebner's figures of *D. simplicifolia* show that the cotyledon in this species has a midrib with two lateral veins near the base, but sometimes the midrib forks at the summit, indicating again the early dichotomy of the cotyledon. In one

of his figures, where there is no forking of the mid-vein, the lateral veins seem to follow the margin of the leaf and rejoin the central vein, thus inclosing two large areoles like those found in the cotyledon of *Kaulfussia;* no mention of this, however, is made in the text. *D. jamaicensis* (fig. 125, *A*) shows a very similar form and vena-
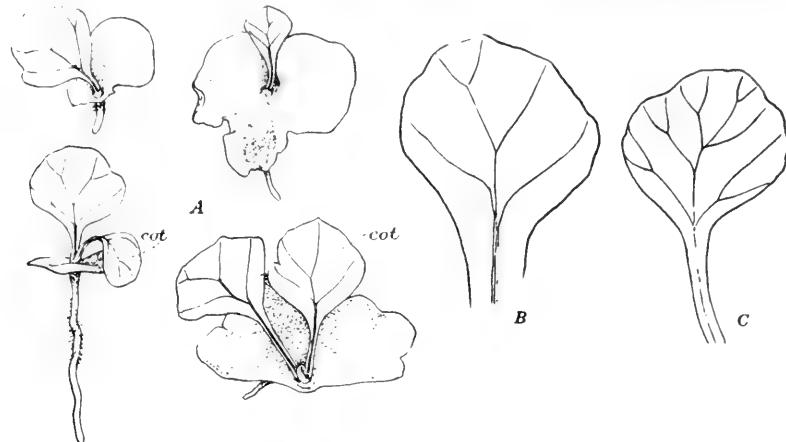


FIG. 125.

A. Four young sporophytes still attached to the gametophyte of *Danæa jamaicensis.* × 2.
B. Young cotyledon of *D. elliptica.* ×15.
C. An older cotyledon of *D. elliptica.* ×3.5.

tion, but the cotyledon is larger and there is sometimes a forking of the lateral veins. In this species also there may sometimes be found a fan-shaped lamina with true dichotomous venation like that of *Marattia*, but as I had little material of this species I can not say how common this form is. It is probable that an examination
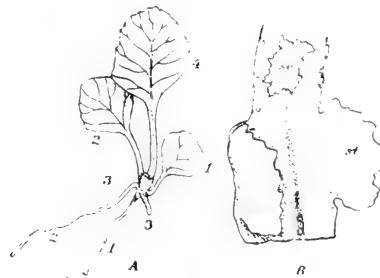


FIG. 126.

A. Young sporophyte of *D. jamaicensis*, with four leaves, slightly enlarged. 1, the cotyledon.
B. Base of the fourth leaf, showing stipules, *st*, and peltate scale, *sc.* × 20.

of a large series of young plants would show examples of the same type of cotyledon as in the other species. The cotyledon of *D. elliptica* (fig. 125, *B*, *C*) is of much the same form as that of *D. jamaicensis*, but the cotyledon is larger as a rule and the venation may be more complex, although conforming to the same general type.

The cotyledon in *Kaulfussia* (fig. 123) is spatulate in form and not unlike that of *Danæa* in shape and also closely resembles in outline the broader leaf forms of *Ophioglossum moluccanum*. The resemblance to the latter is much increased by the venation, which is reticulate and extraordinarily like that of the cotyledon of *Ophioglossum*. As in *Ophioglossum*, there is usually a mid-vein, but this is not, however, noticeably thicker than the laterals. The latter, instead of extending free to the edge of the leaf, are connected at their distal ends with the mid-vein so as to inclose more or less elongated areoles. The central vein does not extend to the tip but, as in *Danæa*, divides into equal branches which join above so as to inclose
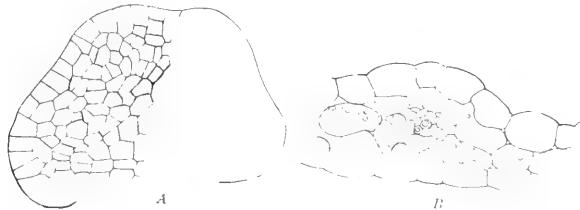


FIG. 127.

A. Lamina of young cotyledon of *Marattia douglasii*.  ×260.
B. Section of lamina of cotyledon.  ×300.

a terminal areole. A careful examination of the secondary veins shows that their divisions are really dichotomous, as in the lateral veins of the other Marattiaceæ, and some of the ultimate branches may reach the margin of the leaf, or more rarely they may end freely within the areoles.

As the cotyledon develops there is more or less elongation of the petiole, which, however, very seldom exceeds the lamina in length. The petiole is usually more or less deeply channeled upon its inner face. In *Marattia douglasii* (fig. 133) the base of the cotyledon is much enlarged just above the level of the stem apex, which is
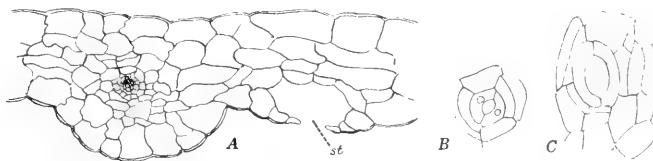


FIG. 128.

A. Section of lamina of cotyledon of *Kaulfussia*, traversing one of the large stomata, *st*.  ×100.
B. A young stoma.
C. An older one.

thus almost inclosed in a sort of cleft in the base of the cotyledon, very much as it is in the case of *Botrychium* or *Helminthostachys*. In *Angiopteris* there is but slight contraction at the base and the stem apex forms a strongly oblique surface, hardly covered at all by the base of the cotyledon. The base of the cotyledon is still more conspicuously hollowed out in *Danæa* and especially in *Kaulfussia* (figs. 136, 137), and in the latter there is a circular ridge completely surrounding the stem apex, exactly as is the case in the young bud in *Ophioglossum moluccanum*. Traces of this ridge can be seen also in *Danæa* and *Marattia*, but this is much less conspicuous than in *Kaulfussia*. In the latter this cavity is especially marked, so that one might almost speak of a stipular sheath, and this resemblance to *Ophioglossum* in the

relation of the leaf base and stem apex, especially in connection with the *Ophio-glossum*-like venation in *Kaulfussia*, is not without significance in its bearing upon a possible relationship between the Marattiaceæ and the Ophioglossaceæ.

In all of the genera the lower part of the cotyledon and sometimes the stem apex are more or less covered with hairs and scales. These are less abundant in *Marattia* and *Angiopteris*, where they are, for the most part, short hairs whose terminal cells contain a great deal of tannin and stain very strongly. In *Kaulfussia*, and especially in *Danæa*, conspicuous scales of peculiar form are abundant.
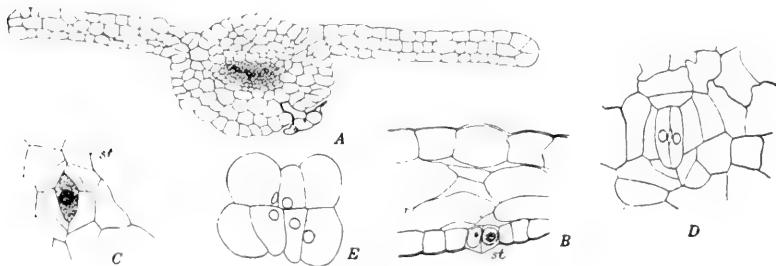


FIG. 129.—*Danæa jamaicensis.*

A. Section near base of lamina of cotyledon. ×75.
B. Part of the lamina. *st*, stoma. ×200.
C. Young stoma.
D. An older stoma. ×200.
E. Cross-section of a peltate scale, from young cotyledon. ×200.

In *Marattia douglasii* the nearly cylindrical petiole is somewhat channeled on its inner side and the single and nearly centrally placed vascular bundle appears almost circular in section. The xylem forms a somewhat crescent-shaped body, completely inclosed by the phloem, but the phloem is less developed upon the inner side and the bundle closely approaches the collateral type. Tannin cells occur, but are not so conspicuous as they are in *Angiopteris*. The lamina of the cotyledon is similar in structure to that of the latter, the leaves differing mainly in the much smaller development of the mesophyll, which is often reduced to a single layer of cells (fig. 127, *B*). The smaller veins have the xylem reduced to a few—one to three—small tracheids situated upon the upper side of the bundles, so that the bundles are strictly collateral. Stomata of the ordinary form occur upon the lower surface of the leaf and these sometimes show a series of concentrically arranged cells about them.



FIG. 130.

A. Section of petiole of cotyledon of *Angiopteris*. ×95.
B. Vascular bundle. ×200. The shaded cell is a tannin sac.

The petiole of the cotyledon in *Angiopteris* resembles in section that of *Marattia*, but it is slightly winged (fig. 130), and the tannin cells adjacent to the vascular bundle are more conspicuous than in *Marattia*.

In *Kaulfussia* (fig. 137, *C*), where the petiole is stouter than in the other genera, the section is more nearly circular than in *Angiopteris*, but it is also slightly winged, these wings being developed almost to the proportions of stipules at the contracted

basal portion. The vascular bundle has a greater development of xylem than in the other genera and the xylem forms a band of considerable extent in the oval section of the bundle. The structure of the bundle is concentric, but, as in other cases, the phloem is better developed upon the outer side. Below the free base of the cotyledon, where the trace enters the stem, there is a reduction of the inner phloem, or the inner phloem entirely disappears, and the bundle there becomes truly collateral. No definite endodermis can be recognized and the tannin cells found in the cotyledon of *Angiopteris* are quite absent.
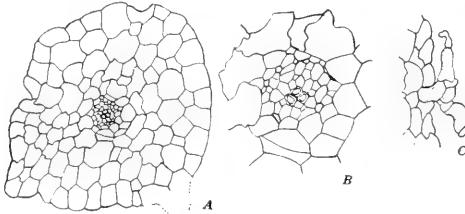


FIG. 131.

A. Section of petiole of cotyledon of *Danæa elliptica*. ×75.
B. The vascular bundle. ×200.
C. Section of a peltate scale. ×75.

The section of the petiole of the cotyledon in *Danæa* (fig. 131) is nearly circular, and the wings found in the other forms are almost entirely obliterated. The ground tissue is composed of undifferentiated parenchyma, with no trace of tannin cells. The vascular bundle is circular in outline, with a small group of three or four tracheids placed somewhat toward the inner side of the bundle, but separated from the outside by about two rows of cells, probably phloem, but not showing any differentiation. Small protophloem elements, staining darkly, can be seen upon the dorsal side of the bundle and an occasional similar cell can be
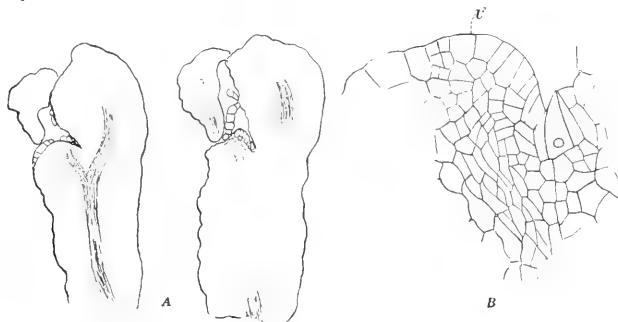


FIG. 132.

A. Two longitudinal sections of a somewhat older sporophyte of *M. douglasii*.
B. Apical region of stem, showing apical cell and second leaf. *l²*. ×200.

seen toward the ventral side, but most of the ventral surface is destitute of these. The petiole broadens at its base, becoming flattened, and in this region the inner phloem disappears and the bundle becomes collateral, as it is in *Ophioglossum*. The tracheary tissue of the cotyledon in all of the Marattiaceæ is composed of slender, reticulately marked tracheids, like those of *Ophioglossum*. Sieve tubes are prob-

ably present in the phloem, but in material mounted in Canada balsam the sieve tubes are very poorly differentiated.

Upon the cotyledon as well as upon the later leaves in *Danæa* very characteristic peltate scales are developed. These arise as single club-shaped cells, the swollen end of which is cut off by a cross wall. In the upper cell, which broadens rapidly, a series of vertical walls arises, sometimes arranged very regularly (fig. 129, *E*). In the case figured there had evidently been two median, intersecting walls, so that the cells were arranged in a somewhat quadrant fashion; more commonly the divisions are less regular and the cells increase considerably in size by further divisions. A section of an older scale may be seen in fig. 131, *C*. Stomata are developed upon the lower surface of the cotyledon in all of the Marattiaceæ. These have already been referred to in the case of *Marattia*, and in *Angiopteris* they are very similar to those found in *Marattia*. In *Danæa*, previous to the formation of the stoma, there are several preliminary divisions, the cells being cut
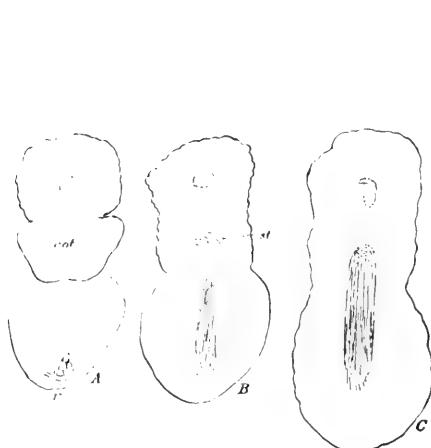


FIG. 133.

Three longitudinal sections of a young sporophyte of *Marattia douglasii*, cut in the plane of the cotyledon, *cot. st*, stem apex; *r*, root apex. ×75.
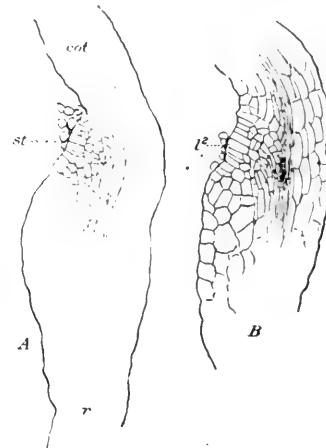
FIG. 134.

Two nearly median sections of a young sporophyte of *Angiopteris. cot*, cotyledon; *st*, stem apex. *l²*, second leaf. ×75. The bundles of the cotyledon and primary root are continuous.

off in a spiral fashion, the last division resulting in the formation of the mother cell of the stoma (fig. 129, *C*). The complete stoma appears, therefore, surrounded by a series of somewhat concentrically arranged accessory cells (fig. 129, *D*). Similar accessory cells, but much less developed, can be recognized in *Marattia* also.

The stomata of *Kaulfussia* have long been known for their great size and the fact that they form permanently open pores, a peculiarity already developed in the stomata of the cotyledon. These are very much larger than those of the other genera and can be readily recognized by the naked eye as little dots. The guard cells become very large (fig. 128) and strongly curved, so that the stoma appears almost circular in outline, with a very large permanently open central pore. This large pore is surrounded by concentrically arranged series of cells, suggesting that the mother cell of the stoma is cut off very much as in *Danæa*, but material was wanting for a thorough investigation of this point.

### THE STEM OF THE YOUNG SPOROPHYTE.

The stem apex projects very little or not at all, and occupies but a limited area close to the base of the cotyledon. Its outer surface is nearly horizontal, except in *Angiopteris*, where it is generally strongly inclined (fig. 134). There probably is always a single apical cell. Segments are cut off from the sides, and very often also inner segments, where the base of the apical cell is truncate. The lateral segments also have, cut off from their inner faces, cells which contribute to the growth in length of the stem, which remains, however, very short in the young sporophyte, the leaves being very much crowded together. As we have already stated, no central cylinder properly belonging to the stem can be demonstrated at this time, all of the procambial
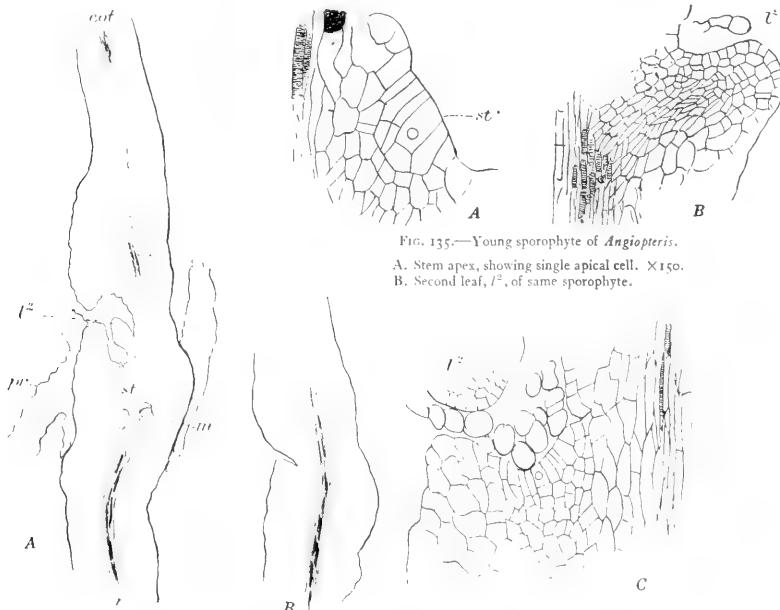


Fig. 135.—Young sporophyte of *Angiopteris*.

A. Stem apex, showing single apical cell. ×150.
B. Second leaf, *l²*, of same sporophyte.

Fig. 136.

A. Nearly median section of a young sporophyte of *Kaulfussia*. *l²*, second leaf; *m*, mucilage ducts; *st*. stem apex; *pr*, gametophyte. × about 20.

B. Another section of same, showing continuity of bundles of cotyledon and root.
C. The stem apex.

tissue belonging either to the cotyledon or to the root. In the older embryo of *Marattia douglasii* the apex of the stem (fig. 139, *B*) is occupied by a group of relatively large cells, which at first sight seem to be pretty much alike; but a careful examination of these makes it probable that one of them may be considered as the real initial cell. This, in cross-section, appears almost square and shows a fairly regular segmentation. While it must be said that the segmentation is not so regular that one can assert without question that all of the meristem is really due to the activity of a single cell, I believe that this is probably the case.

My own investigations of the stem apex in *Angiopteris* have led to the conclusion that there is a single initial cell here also in the young sporophyte, although Farmer

in his earlier studies of this plant failed to find a single initial cell; however, in a more recent paper (Farmer 3) he has decided that a single apical cell is present in the young sporophyte of *Angiopteris*, and he gives figures of this. In longitudinal section (fig. 134) the apical cell appears oblong, with a markedly truncate base, while in transverse section it is imperfectly triangular in outline. The apical cell of the stem in *Kaulfussia* (fig. 136, C) is usually somewhat broader than it is in *Angiopteris*, and closely resembles that of *Ophioglossum moluccanum*. It has a broadly truncate base and is somewhat narrowed above. In transverse section it approaches the triangular outline of the apical cell in *Ophioglossum*, but the segmentation is less regular and in this respect it is not unlike that of *Angiopteris*. The stem apex was examined in three species of *Danæa*, all of which seemed to agree
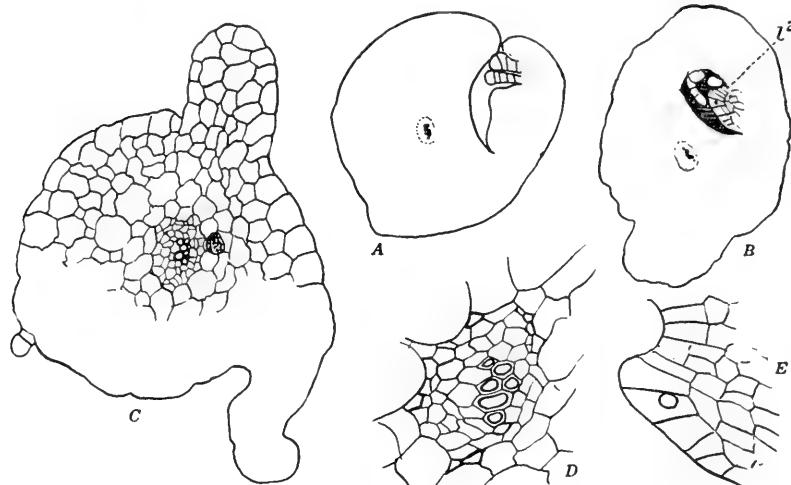


FIG. 137.—*Kaulfussia*.

A, B. Two transverse sections of a young sporophyte, above     C. Transverse section of petiole of cotyledon.   ×90.
level of stem apex, showing closed sheath formed by     D. Vascular bundle of cotyledon, more enlarged.
base of cotyledon. $l^2$, second leaf.   ×50.          E. Second leaf.

closely in the form of the apical cell, which is much more definite in *Danæa* than it is in any of the other genera. Brebner's account and figure of the apical cell in *D. simplicifolia* agree closely with my own preparations of *D. elliptica*, *D. jenmani*, and *D. jamaicensis*.

The apical cell (fig. 140, *A*), seen in longitudinal section, is very deep and, unlike that of the other genera, is not usually, at least, truncate below, but pointed, so that it closely resembles in form that of *Botrychium* or *Helminthostachys*. In transverse section it also usually appears triangular, so that it is tetrahedral like that of *Botrychium*. While the segmentation is somewhat less regular than in *Botrychium*, nevertheless the limits of the younger segments can be followed without much difficulty, especially in longitudinal sections, although the exact sequence of the divisions in the segments themselves did not seem to be absolutely regular.

In all of the species the tissue derived from the inner cells of the segments of the apical cell, or cut off from the base of the apical cell itself, remains as undifferentiated parenchyma and contributes only to the central pith of the stem. Except

that the leaves arise from the segments of the apical cell of the stem, and their tissues are therefore indirectly derived from the stem apex, we may say that the stem apex takes no part in the differentiation of the fibro-vascular system, which in the young sporophyte is composed entirely of the leaf and root traces.

### THE ROOT.

Only a small number of sections of the primary root were obtained in *Marattia douglasii*, and these showed some variation, so that it is not possible to state positively what is the typical form of its apical cell, but there is no question that the primary root grows from a single initial cell, as in the other genera. In longitudinal section it appears oblong, with a broadly truncate base from which segments arise, as well as from the lateral faces. There seem to be regularly four sets of lateral
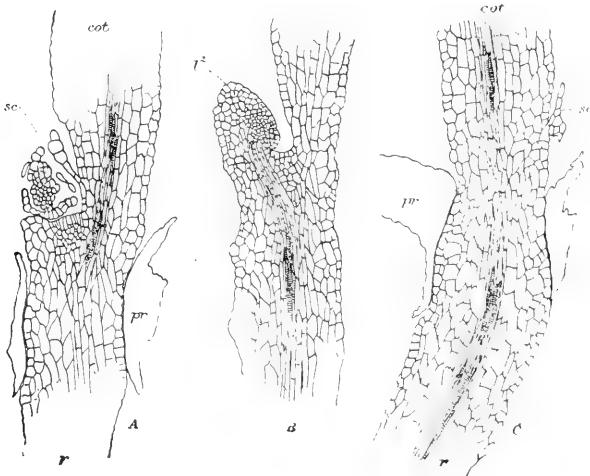


Fig. 138.

A. Nearly median section of a young sporophyte of *Danæa jamaicensis*, passing through stem apex. × about 40.
B. Another section of same, passing through second leaf, $l^2$
C. Section of another similar sporophyte cut at right angles to that shown in A and B, and showing continuity of bundles of cotyledon and primary root. *pr*, gametophyte; *sc*, scales.

segments, although cross-sections of the apical cell sometimes appear almost triangular. The root cap is formed in part from segments derived from the outer face of the apical cell, in part from similar segments cut off from the outer portion of the youngest lateral segments of the apical cell. The central cylinder of the root is formed in part from the basal segments of the apical cell, but the lateral segments also contribute to it (fig. 141, *D*).

In *Angiopteris* (fig. 111, *C*) the apical cell in the earlier stages of the root may appear triangular in longitudinal section, but in the later stages it is usually more or less truncate.

The primary root in *Kaulfussia*, in the few cases where satisfactory sections were made of it, did not show the tetrahedral apical cell, but this was truncate at the base and in cross-section appeared four-sided (fig. 142). In this respect it seems to approach *Marattia*. The primary root in all of the Marattiaceæ is ordinarily diarch;

the statement (Campbell **3**, page 14) that in *Marattia douglasii* the primary root is tetrarch I have found to be based upon a mistake, the root which was so described not being the primary root, but evidently one of the later ones. I have



occasionally found very strong plantlets in *Danæa*, where the primary root was triarch, as it sometimes is in *Botrychium*, and very commonly is in *Helminthostachys;* but such specimens are not common. The root bundle is bounded by a conspicuous endodermis, whose cells show very plainly the characteristic radially thickened bands (figs. 143, *en*; 144). The endodermis gradually loses its definite appearance at the junction of the root and leaf bundles, and I have not been able to recognize it above the level of the base of the cotyledon, although it is not at all impossible that with proper staining it might be traced further up.
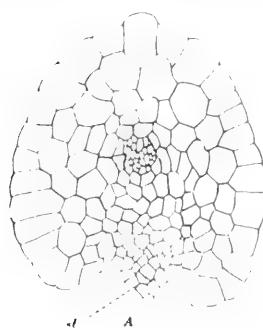
FIG. 139.

A. Transverse section of a young sporophyte of *Marattia douglasii*.
   *st*, stem apex.   ×150.
B. The stem apex.   ×360.

### THE SECOND LEAF.

The second leaf begins to develop while the cotyledon is still small, and by the time the latter ruptures the overlying prothallium tissue the second leaf is clearly evident as a flattened cone, closely resembling the early stage of the cotyledon. In *Kaulfussia* the second leaf arises quite close to the cotyledon and on the same side of the stem apex, so that the dorsiventral character of the stem is already indicated at this early stage of development. In the other genera the second leaf arises nearly opposite the cotyledon, but not
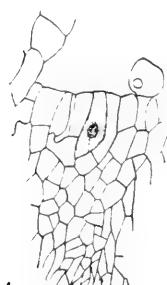
FIG. 140.

A. Stem apex of a young sporophyte of *Danæa jamaicensis*, showing single apical cell.   ×180.
B. Second leaf from same.
C, D. Sections of primary roots, showing apical meristem.   ×180.
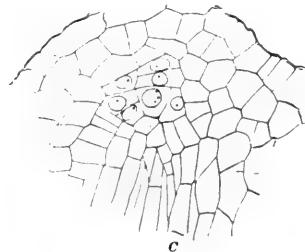


exactly so, and the subsequent leaves form a spiral, and for a time at least, except in *Kaulfussia*, the anatomy of the shoot is radial. This radial arrangement is retained permanently in *Marattia* and *Angiopteris* and also in some species of

*Danæa*, e. g., *D. elliptica;* but in *D. jenmani* and in *D. jamaicensis*, as well as in most of the other species, the stem finally becomes dorsiventral, as it is from the first in *Kaulfussia.*

The development of the second leaf is marked by an increased activity in the apical meristem, quite close to the apical cell, and it is clear that the leaf arises from the younger segments, but it is impossible to state exactly what the relation of the young leaf is to the younger segments of the apical cell, or to say whether the whole
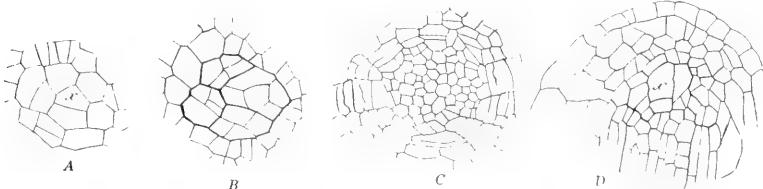


Fig. 141.

A–C. Three transverse sections of primary root of *Marattia douglasii*.
D  Longitudinal section of apex of primary root.

of the leaf is the derivative of a single segment.  When the young leaf can first be distinguished it is elevated very slightly above the level of the stem apex and its growing point is composed of a group of columnar cells.  Sometimes, especially in *Danæa*, there may be recognized near the center of this group of cells a single, somewhat larger one, which may be interpreted as a single apical cell, but this is not always recognizable.
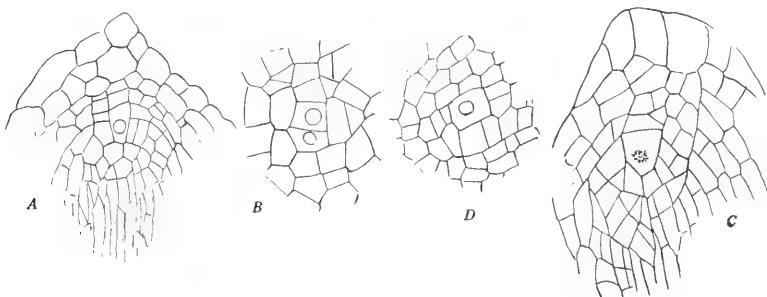


Fig. 142.

A, longitudinal, B, transverse, sections of apex of primary root of *Kaulfussia*.  ×200
C.  Apex of second root.
D  Transverse section of root apex.

Even before there is any noticeable projection of the leaf rudiment, there is developed beneath it the beginning of the short strand of procambium which is to form its vascular bundle, and this can be followed downward to a point below the stem apex, where it joins the trace from the cotyledon.  Above this junction there is no evidence of procambium tissue extending into the stem apex, but the whole tissue lying between the junction of these two primary vascular bundles and the apical meristem of the stem is made up of undifferentiated parenchyma.

The outer surface of the stem apex may be nearly horizontal or (especially in *Angiopteris*) it may be strongly inclined and a single fairly conspicuous initial cell

may usually be recognized without difficulty. This cell is usually quite deep and in all of the genera except *Danæa* has a truncate base in longitudinal section. In *Danæa*, however, the base is usually pointed and the cell closely resembles the corresponding cell in *Botrychium*, or the typical leptosporangiate ferns. The growth of the stem is usually rather slow and the leaves of the young plant are much crowded.

In all of the forms examined, the tissue derived from the inner cells of the segments and from the basal segments of the apical cell, where such segments were formed, remained as undifferentiated parenchyma and contributed only to the internal pith of the stem. Except that the leaves arise from the segments of the apical cell of the stem and all of their tissues are therefore indirectly derived from it, the stem apex of the young sporophyte takes no part at all in the development of the fibrovascular system of the stem, which, except for the bundles of the roots, is entirely composed of the leaf traces.

The second leaf in *Angiopteris* usually shows a pretty well-defined but slender midvein, which may, however, fork at the apex, and this is true also of the third leaf. It is in the third leaf, both in *Marattia* and *Angiopteris*, that the characteristic stipules are first developed. These appear as lateral wing-shaped bodies close to the
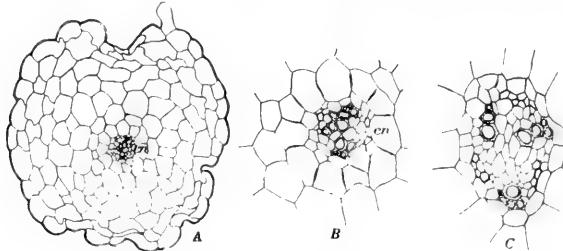


FIG. 143.

A. Section of primary root of *Danæa jamaicensis*.  ×70.
B. The vascular bundle.  ×175.  *en*, endodermis.
C. Triarch bundle from a primary root of *D. elliptica*.

base of the petiole, partially inclosing the next youngest leaf and the stem apex. The third leaf in *Angiopteris* and several others succeeding it assume a more and more pronounced lanceolate form with a prominent midrib and pinnately arranged lateral veins which usually fork once. The margin of the leaf is serrate. After several of these lanceolate leaves have been developed, leaves are formed in which the base is provided with auricles, and these gradually pass into the next type of leaf, which is ternate, the terminal leaflet being very much larger than the lateral ones (plate 13, figs. 4, 5). These ternate leaves then pass by intermediate stages into the pinnate form of the leaves of the adult plant.

In *Kaulfussia* the second and third leaves closely resemble the primary one, except that they are somewhat larger (fig. 123). The second leaf develops well-marked stipules which are found in all of the later leaves. The earlier leaves do not show a definite midrib, though there is usually a delicate midvein at the base of the lamina, but this vein forks usually about half-way up. In the older leaves (fig. 123, *C*) there is developed a stout midrib, from which extend distinct pinnately arranged lateral veins, much as in *Angiopteris*, but these lateral veins are connected by a system of anastomosing veins, so that the leaf most strikingly resembles that of a typical Dicotyledon. It is not until a very late period that the leaves in *Kaulfussia* assume the compound form of the older plant (plate 11, fig. 1).

In *Danæa* a dozen or more leaves may be formed before there is any branching of the lamina. While the cotyledon may show an approach to the pinnate venation of the older leaves, there is almost always a dichotomy of the apex of the slender midvein, and this is generally true also for a number of the subsequent leaves, although in these later leaves the midrib becomes well marked and the lateral veins are developed apparently monopodially. In *D. simplicifolia* the leaves remain permanently unbranched, but in all of the other species the later leaves are once pinnate. The intermediate leaves have the ternate form found in *Marattia* and *Angiopteris*, and after a few of these, about two or three, have been formed, five foliate leaves appear and the number of leaflets gradually increases, as new leaves are formed, until the full number of leaflets is developed. The early ternate leaves often have the terminal leaflet much larger than the lateral ones, and the leaf
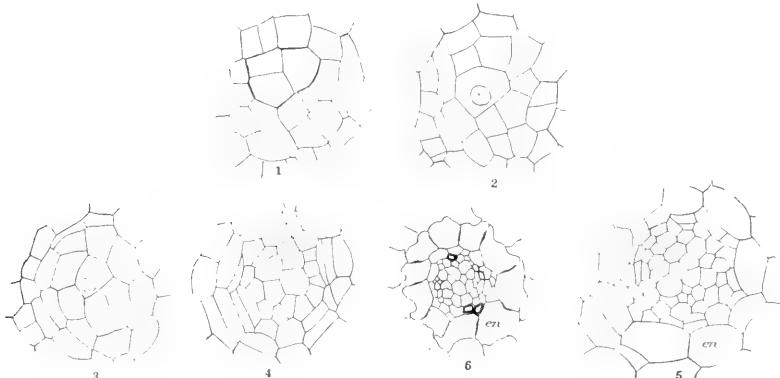


FIG. 144.

1-5. Transverse sections from a series of a young root of *Danæa jamaicensis*. 2 shows apical cell.
6. Section of bundle from fully developed primary root. *en*, endodermis.

closely resembles the corresponding stages in *Angiopteris*. The petiole in most species is more or less obviously winged. In *Danæa* the stipules are (sometimes, at least) not formed until the fourth leaf. In the fourth leaf (fig. 126, *B*), however, they are well developed, appearing as two conspicuous wing-like organs with serrate edges.

In all of the genera there is a root formed for each leaf in the earlier stages of the sporophyte, but how late this continues was not determined. In the older sporophyte more than one root may be formed for each leaf. In *Kaulfussia* the second root is developed earlier than in the other genera, beginning to develop while the second leaf is still very small, and in general the roots in *Kaulfussia* seem to develop earlier than in the other genera. The second root is especially late in developing in *Danæa*, the very first rudiment of the root making its appearance between the first and second leaves, after the latter is already quite well advanced.

## III. THE OLDER SPOROPHYTE.

The vascular system of the adult sporophyte in the Marattiaceæ is extremely complex, especially in the larger species of *Marattia* and *Angiopteris*. In *Danæa* and *Kaulfussia* it is simpler, but in these also the arrangement of the bundles in the adult stem is by no means easy to trace. A thorough study of the condition in the early stages of the sporophyte is therefore specially important for understanding the much more complex arrangement of the bundles in the older plant.

In view of the conditions that we have found to exist in the Ophioglossaceæ, and particularly the nature of the vascular system in the young sporophyte of *Ophioglossum moluccanum*, a careful reinvestigation of the developmental history of the vascular system in the young sporophyte of the Marattiaceæ, especially the simpler and presumably more primitive genera *Danæa* and *Kaulfussia*, was very much needed. To this end series of sections were made of young sporophytes of both of the latter genera, ranging from individuals in which the second leaf was still undeveloped to those in which a dozen or more leaves had been formed. These series were fairly complete and made it possible to trace quite satisfactorily the evolution of the vascular system in the young sporophyte of both *Danæa* and *Kaulfussia*. Brebner (Brebner 3) has given an accurate account of the distribution of the bundles in the young sporophyte of *Danæa simplicifolia*, which agrees quite closely with the arrangement of the bundles as I have found them in the species studied by me. Jeffrey (Jeffrey 3) has described and figured a few specimens of *D. alata* (?), but his material was too incomplete to make the study at all satisfactory.

The available material of *Marattia* was much less complete, although a number of stages were secured. Farmer has made an exhaustive study of the vascular system in the young sporophyte of *Angiopteris* (Farmer 3), and an extended study of *Angiopteris* did not seem necessary, therefore, except for a study of the early stages to determine the origin of the vascular bundles. All of the writers mentioned concerned themselves almost exclusively with the fully developed bundles, beginning their study at the base of the stem and working upward. No attempt was made to follow out the development of the vascular bundles from the meristematic region of the apex and proceeding downward, a method of study which we think would have materially modified the conclusions drawn by these observers in regard to the relation of the different parts of the vascular system.

In my studies as to the origin of the vascular system in the young sporophyte of the Marattiaceæ the course of the bundles has been carefully followed in series of microtome sections, passing downward from the apical meristem to the base of the sporophyte, where the axial primary bundle passes into the primary root. These series of transverse sections were then compared carefully with series of longitudinal sections of young plants of approximately the same age, and in this way a clear conception was obtained of the arrangement of the vascular system within the stem of the young sporophyte.

### THE DEVELOPMENT OF THE VASCULAR SYSTEM IN DANÆA.

Especial attention has been given to the development of the vascular system in *Danæa* and *Kaulfussia*, as these have received less attention than *Marattia* and *Angiopteris*, and moreover are probably more primitive forms whose structures have departed less from the ancestral type than those of either *Marattia* or *Angiopteris*.

A longitudinal section of the young sporophyte of *Danæa jamaicensis* at the period when the root is just about to emerge is shown in fig. 121, *A*. The root in this species, as in *D. simplicifolia*, described by Brebner, is the first organ to appear

outside the prothallium; the cotyledon at this time is still very small and the lamina is scarcely developed. The root apex does not show in the section figured and all that can be seen is the upper portion of its stele, which joins the two young vascular bundles that extend respectively into the cotyledon and the second leaf, which can already be recognized. The greater part of the young sporophyte is made up of large thin-walled cells. Figure 121, $B$, shows a median section of the young cotyledon from this same embryo, which is strongly bent over the stem apex. It is possible that the square terminal cell, which in the drawing has the nucleus indicated, is the apical cell of the leaf, but this is not at all certain. The outer part of the cotyledon is made up of large thin-walled cells like those which compose the bulk of the sporophyte, but at its apex there is active cell division, and traversing the young cotyledon and placed somewhat toward its inner side, can be seen the primary vascular bundle, made up of elongated procambium cells. This bundle can be easily followed down into the body of the sporophyte, where, as we have seen, it is
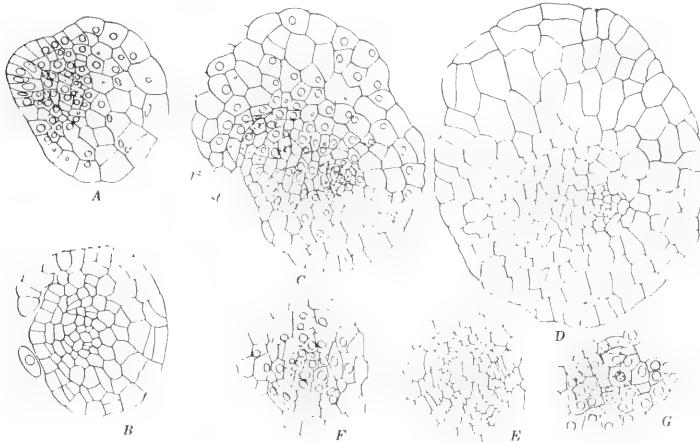


FIG. 145.

Transverse sections of a very young sporophyte of *Danæa jamaicensis*. ×150.
A shows cotyledon apex; C, apices of the stem and second leaf; G shows root apex.

continued without interruption into the stele of the primary root. The stem apex, which does not show in the figure, is in a slight depression close to the base of the cotyledon and shows, as usual, a single deep apical cell. Nearly opposite the cotyledon is the beginning of the second leaf ($l^2$); this is shown in median section in fig. 121, $B$. The group of narrow columnar cells that compose its apex does not show a recognizable single initial cell. A short distance below its apex, the procambium of its vascular bundle becomes differentiated and is continued downward until it joins the bundle from the cotyledon. The tissue arising above the junction of these two bundles and continuing upward into the apex of the stem is composed of absolutely undifferentiated parenchyma and there is no evidence at all of any stelar tissue in the stem above the junction of these two primary leaf traces; indeed, the stem apex lies decidedly on one side of the plane which traverses the vascular bundles of the two leaves. Fig. 145 shows a number of cross-sections from a series taken from a large embryo of *Danæa jamaicensis*, before the emergence of the cotyledon ($A$),

11

whose apex is still undivided. *C* shows the apices of the stem and second leaf, the former being shown on a larger scale in *G*. The section of the cotyledonary bundle, lying to the right of the stem apex, is plainly evident in section *C*. *D* shows the separated bundles of the cotyledon and very young second leaf, which lower down unite into a single strand.

Whether we can properly speak of a stem at all in connection with the tissues composing the central region of the young sporophyte is questionable. It is perfectly evident that this central region is composed partly of tissue belonging to the base of the cotyledon, which is in no sense to be looked upon as an adjunct of the
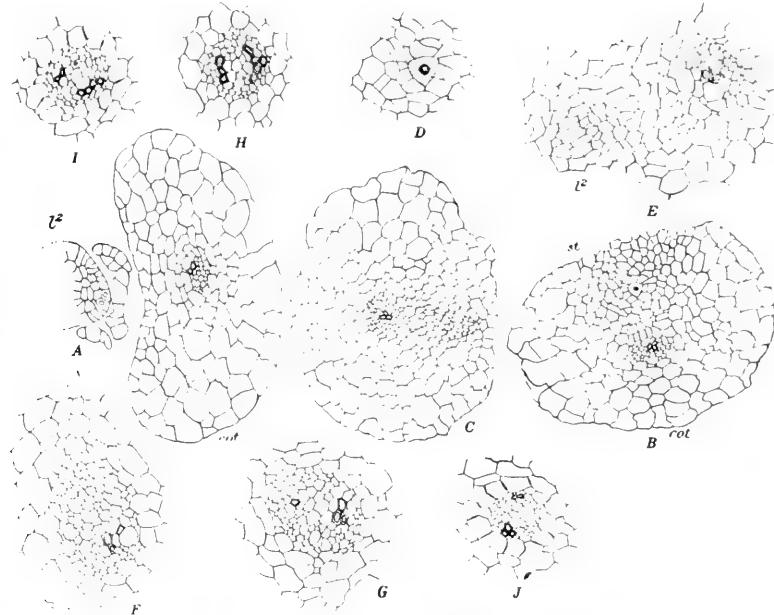


FIG. 146.—Ten transverse sections of a young sporophyte of *Danæa jamaicensis*.

A-C. Three sections near the stem apex, which is shown in B    F-J. Show the condition of the vascular strand in the lower
    and more highly magnified in D.  Fig. C is reversed in        region of the sporophyte.
    position with reference to A.                              H, I. Are in the transition region.
E. The central region of C.                                    J. Shows the root stele.   ×200.

stem, and partly of the root base; the two structures, i. e., the bases of the root and the cotyledon, merge insensibly into each other. To this tissue, derived from the root and the cotyledon, must be added a large amount of superficial tissue derived originally from the foot of the embryo. In speaking of the axis of the young sporophyte as a "stem" it must therefore be borne in mind that this stem is really a composite structure, partly leaf, partly root, and partly foot.

Fig. 138, *A* and *B*, shows two longitudinal sections of an older stage after the emergence of the cotyledon. *A* passes through the stem apex and it is evident that there is no vascular bundle extending into the stem. The tracheary tissue is now well developed in the leaf trace. *B* shows the trace from the second leaf at its point of junction with the bundle from the cotyledon. In fig. 138, *C* is a similar

stage to that shown in $A$, but cut at right angles to it, so as to show the continuity of the bundles of the cotyledon and primary root, which together constitute the primary axial vascular bundle of the sporophyte, as they do in $Ophioglossum\ moluccanum$. The similarity of such a section as that figured to the similar stage in $O.\ moluccanum$ is sufficiently obvious. The stem apex is shown in fig. 140, $A$, and the rudiment of the second leaf is shown in fig. 140, $B$.

Figure 146 shows ten transverse sections from a series made from a young sporophyte of $D.\ jamaicensis$ of almost exactly the same age as that shown in fig. 138, $B$. $A$ is cut above the level of the stem apex and passes through the cotyledon and apex of the second leaf. The arrangement of the cells at the apex of the latter suggests the possibility of a single apical cell. The base of the petiole of the cotyledon is much flattened and is made up of large, thin-walled parenchyma with no traces of tannin cells or mucilage ducts. The vascular bundle is oval in outline and decidedly collateral in structure. There is no recognizable endodermis, and I shall not attempt to say whether the cells immediately surrounding the bundle should be spoken of as endodermis or pericycle. Except for two small tracheids on the inner side of the bundle the vascular bundle is composed of delicate tissue, apparently parenchyma, but some of the larger elements undoubtedly represent sieve tubes. Brebner states that the sieve tubes in $D.\ simplicifolia$ (Brebner 3, page 524) do not show clearly in sections mounted in Canada balsam, but when examined in other media they show thick, glistening walls and pitted areas. This point was not further examined in the species under consideration, but would probably agree with the observations made by Brebner upon $D.\ simplicifolia$.

Section $B$ passes through the stem apex, whose apical cell is shown on a larger scale in $D$. The cotyledon trace in this section shows three tracheids. In $D$ the section is made a short distance below the stem apex and shows the second leaf trace bending in to join the trace from the cotyledon. The central part of the section is shown more enlarged in $E$, and it is perfectly clear that the region immediately below the apex and lying between two leaf bases shows nothing which can be interpreted as a cauline stele. $F$ and $G$ show sections taken still further down, illustrating the coalescence of the two leaf traces to form the single axial stele. In $G$ there is seen the first tracheid belonging to the leaf trace from the second leaf, and in $H$ the central bundle is complete and there are seen the two xylem masses corresponding respectively to the two leaf traces. One of these, belonging to the primary leaf trace, is more developed than the other one. The larger xylem mass in this case had five tracheids and the smaller one three. $I$ and $J$ are transitional stages between the bundle of the cotyledon and the root, and, proceeding downward, the bundle becomes somewhat smaller and there is a reduction in the number of tracheids, which, however, are plainly seen to be continuous in their development with the two xylem masses in the central bundle of the stem, formed by the junction of the two primary leaf traces, which maintain their identity until they merge into the two xylem masses of the diarch root. Within the root itself the endodermis surrounding the bundle is very conspicuous. The cells making up the endodermis are of large size and the characteristic markings upon their radial walls are very easily seen. The limits of the endodermis become much less clear in the transitional region, and higher up it is difficult to make out a clearly defined endodermis. Brebner figures a very irregular endodermis in the stem of $D.\ simplicifolia$, and perhaps we may assume that a proper endodermis is present also in the young stem of $D.\ jamaicensis$, but it is certainly very inconspicuous, to say the least, in the central region of the young sporophyte, in strong contrast to the very conspicuous endodermis in the root. Farmer (Farmer 3) was able by treatment with sulphuric acid

to demonstrate an external endodermis in the stem bundles of *Angiopteris*, *Marattia*, and *Kaulfussia*, and presumably similar treatment would show its presence in the young stem of *Danæa*.

It is evident that we can not speak of the young sporophyte in *Danæa jamaicensis* as possessing in its early stage a "protostele," or "haplostele," to use Brebner's terminology, unless we choose to call the single leaf trace of the cotyledon a protostele. Just so soon as the two first leaf traces unite, the axial vascular bundle of the young sporophyte has two quite independent xylems.

Figure 147 is a longitudinal section of a sporophyte of *D. elliptica*, slightly older than that of *D. jamaicensis* shown in fig. 138. The second leaf ($l^2$) had already begun to develop the lamina, whose apex was forked so that there were two veins present. A longitudinal section of one of the two leaf lobes is shown in *D*. The stem apex closely resembles that of *D. jamaicensis*, the apical cell having much the same form and size. The stem apex does not lie in the same plane as the leaf traces
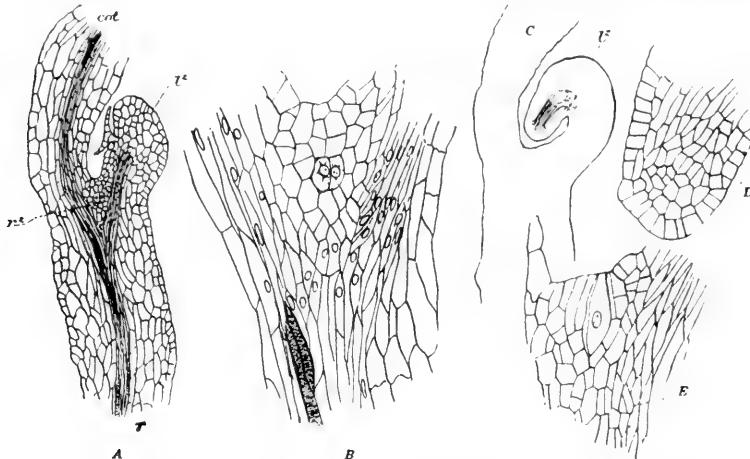


FIG. 147. - *Danæa elliptica*.

A. Nearly median section of a young sporophyte, showing origin of second root, $r^2$.  ×35.
B. Central region.  ×150.
C. Median section of second leaf.  ×35.
D. Apex of second leaf.  ×150.
E. Stem apex.  ×150.

and hence does not show in the figure, which is a section passing directly through the leaf traces. This plant showed very clearly the very earliest stage of the second root (fig. 147, *A*, $r^2$). The apical cell of the young root is cut out from one of the cells of the parenchyma below the stem apex, lying almost exactly half-way between the two primary leaf traces. Only a single segment had been cut off from the apical cell in this case and both the apical cell and this primary segment were easily distinguished from the surrounding tissue by their denser contents. There is nothing to indicate that the cell which had assumed the function of the root initial was in any way essentially different from the neighboring cells. If it is an endodermal cell there is no way of recognizing this, as the endodermis could not be recognized in this part of the sporophyte. Figure 147, *B*, shows an enlarged view of this young root initial, lying above the junction of the two primary leaf traces. Conspicuous reticulate tracheids with pointed ends are present in the trace from the cotyledon, but as yet no permanent tissue has been developed in the bundle from the second leaf.

A somewhat older sporophyte from *D. jamaicensis* is shown in fig. 148. In this plant the third leaf was plainly visible and the second root had developed into a short conical body which was pushing its way through the tissues between the
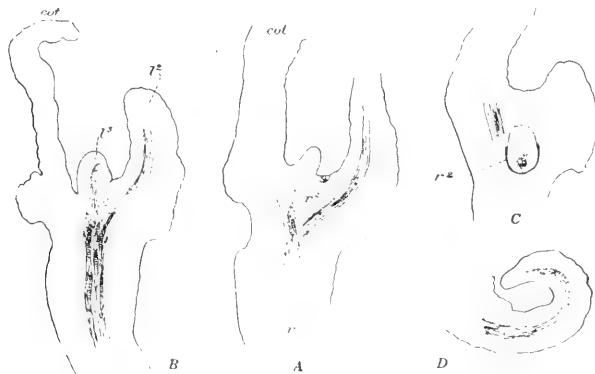


FIG. 148.

A, B, C. Three sections of a sporophyte of *Danæa jamaicensis* with three leaves
D. Median section of the second leaf. In C is shown the second root, $r^2$. ×20.

insertion of the first and second leaves. By this time tracheary tissue has developed in the bundle from the second leaf, and now for the first time we note the presence of large tannin cells in the neighborhood of the vascular bundles. Three of these are shown in fig. 149, lying next the bundle of the second leaf. The irregular ring



FIG. 149.

A, the stem apex; B, apex of the second root of the sporophyte shown in fig. 148. ×150.
The shaded cells in A are tannin sacs.

of narrow cells shown in the figure and lying below the apical meristem marks the point of insertion of the second root. Figure 149, *B*, shows a nearly median section of the apex of this second root. The apical cell is nearly oblong in form and the lateral segments are very large and periclinal segments which contribute to the root

cap are cut off from them. The outer cells of the root cap are very much enlarged and there is an evident space between the root and the surrounding tissues. The trace from the third leaf joins that of the second one close to its union with the bundle from the cotyledon (fig. 148, *B*).

In fig. 150 are shown nine cross-sections from a series made from a plant of *D. jamaicensis*, in which four leaves were evident. A microscopic examination of the stem apex showed, however, that a fifth leaf was also present, which stood nearly directly opposite the cotyledon. *A* shows sections only of leaves 3 and 4, the latter having the stipules conspicuous, while these are absent or scarcely developed at all on the first three leaves, at least in the specimen in question. Between the leaves are seen sections of the numerous scales which beset the surface of the young leaves. *B* is taken somewhat lower down and includes a section of leaf 5. *C* passes through the stem apex and shows clearly the spiral arrangements of the first five leaves, each of which has in its petiole a single concentric bundle which becomes larger in
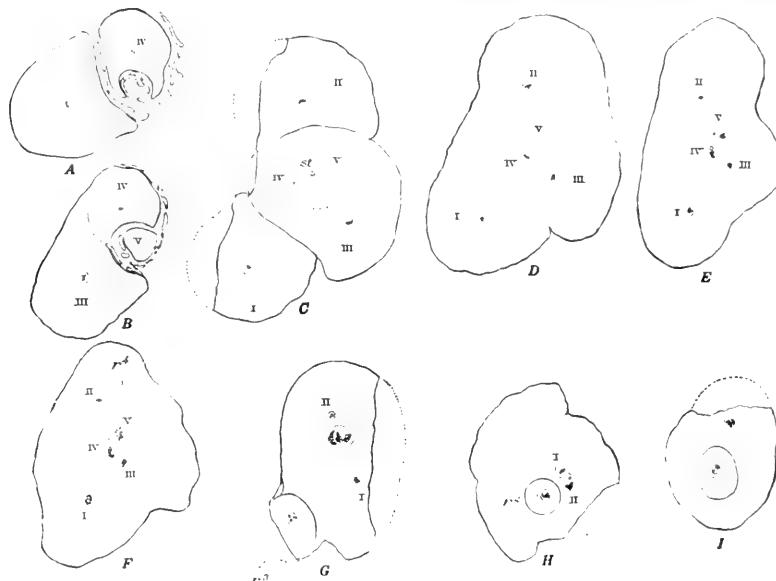


Fig. 150.—*D. jamaicensis*.

Series of transverse sections of a young sporophyte, with three fully-developed leaves and two younger ones. ×20. Section *C* passes through the stem apex. Roman figures indicate successive leaves.

each successive leaf, with a corresponding increase in the development of the xylem. As the sections are examined, farther and farther down in the stem (*D* to *F*), one can see very clearly the way in which the single bundle in the lower part of the stem owes its origin to the coalescence of the leaf traces. Proceeding downward the traces of leaves 4 and 5 are seen to approach gradually and finally to become completely coalescent; and still further down (*F*) the leaf trace from 3 joins that from 4, and a single bundle results, crescent-form in section, but showing clearly its compound nature. The three xylems never become entirely confluent.

For a long time sections of the stem show this single central bundle of crescentic form, at first derived from the coalescence of the third, fourth, and fifth leaf traces, but continued upward in the same form and added to by the addition of the traces from the subsequent leaves. This crescentic stele, which, for convenience may be spoken of as the stele of the stem, is entirely of foliar origin. The crescent never becomes completely closed and its opening in the earlier stages of development can not be properly called a foliar gap. The parenchyma which is inclosed within its curve belongs from the first to the ground tissue and is not part of the stele. Some of the surrounding cells show traces of the typical endodermal markings and it is perhaps safe to say that the stele is bounded by an endodermis, as Farmer states is the case in *Angiopteris* and Brebner in *D. simplicifolia*. The limits of the endodermis, however, especially upon the concave side of the stele, are very vague. The stele, after the complete fusion of the three leaf traces, may perhaps best be described
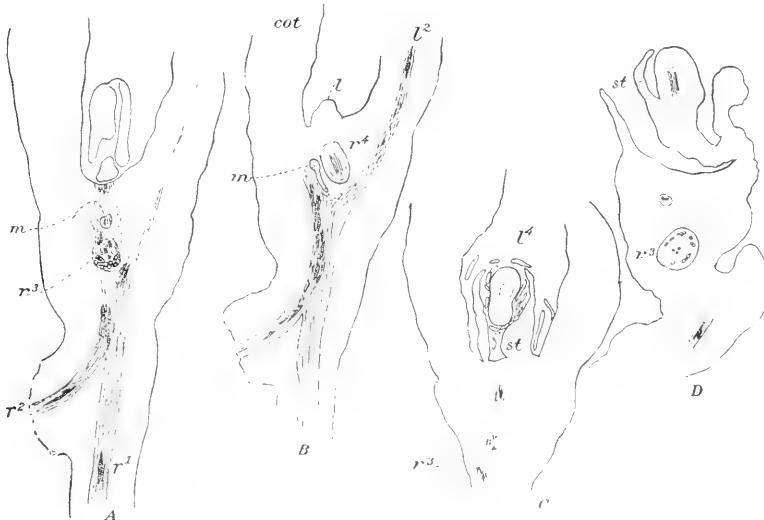


Fig. 151.

Four longitudinal sections of a young sporophyte of *Danæa elliptica*. ×18. The fourth leaf, $l^4$, has the stipules well developed; $r^3$, sections of the third root. *m*, mucilage duct; *st*, stipules.

as concentric in structure, with phloem developed all around the xylem, but there are probably traces of phloem also between the three xylems which represent the three confluent leaf traces.

In the older portions of the stem the bundles become still more completely fused and the compound bundle is oval in outline, but still shows plainly the three xylems of its constituent leaf traces (fig. 150, *G*). In this region the endodermis is rather better developed than it is higher up, but its limits are still rather vague. At this level the traces of the leaves, 1 and 2, are still free, but have approached nearer to the central bundle than is the case higher up.

Still lower down, the trace from the second leaf joins the bundle formed from the later leaf traces, which no longer clearly shows the separate xylems, but approaches the condition which has been described as "protostelic," or "haplostelic," using Brebner's terminology. The xylem elements, however, do not form a solid

core, but are more or less scattered, with thin-walled elements between. After
fusion of the second leaf trace with this is complete, the section of the stem shows
only two bundles, representing the two first leaf traces, and finally (fig. 150, *H* and *I*)
the section of the stem shows the condition already described for the sporophyte
with but two leaves. These finally merge into a single primary axial bundle (*I*),
which, followed downward, merges imperceptibly into the stele of the primary root.
Near the middle of these lower sections can be seen a section of the second root,
which pursues a downward course through the cortical tissue for a very long distance,
but finally emerges and grows downward, side by side with the primary root. The
third root (fig. 150, *G*, *r'*) emerges much higher up and breaks through the cortex
at about the level of the junction of the three youngest leaf traces.

Fig. 153 shows the details of the central tissue from the same sporophyte as
that just described. *A* passes through the stem apex and shows the apical cell cut
somewhat obliquely, and near it the section of the trace from the fifth leaf. *D* is a
section of the fourth leaf trace from the same level. This shows but two tracheids
at a point near the inner limits of the bundle, while on the outer side there is a con-
spicuous curved line of protophloem cells. *B* is a section taken somewhat lower
down and shows the bending in of the leaf trace as it descends into the stem. This
is still more marked in the fifth leaf trace, which, at this level, has a crescentic form,
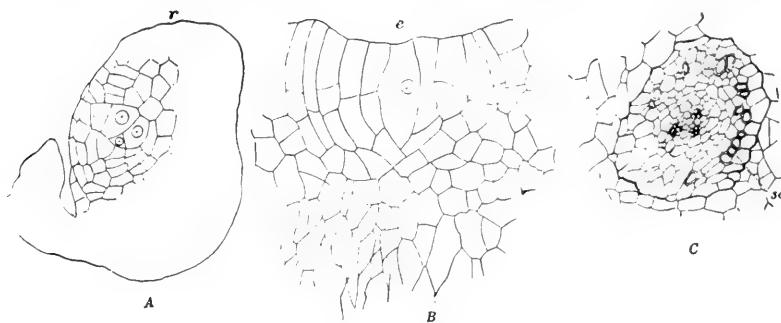


FIG. 152

A. Apex of fifth leaf from sporophyte shown in fig. 151. ×150.
B. Stem apex of same.
C. Base of third root, showing triarch bundle and ring of sclerenchyma in cortex. ×90.

but no permanent elements yet developed. It is probable that a portion of this
crescentic mass of procambium represents the sixth leaf trace. The fourth leaf
trace at this point shows three tracheids which are decidedly larger than those
higher up, and still lower down the tracheids increase still more in size and several
tannin cells can be seen in contact with them. The section of the fourth leaf trace
is connected with the large but undifferentiated trace of the fifth leaf by an isthmus of
procambium cells, so that the sections of the two bundles form the crescent-shaped
section, similar to that seen lower down (fig. 153, *C*). The tracheary elements of the
fused portion of the fourth leaf trace are noticeably larger again than those in the
free portion of the leaf trace. We now notice, for the first time, traces of the mucilage
ducts which afterwards become so conspicuous in the stem. These first appear in
section as rounded cells (*m*), and it is evident, as Farmer has shown in *Angiopteris*,
that the earliest mucilage canals are of lysigenous origin, i. e., they are formed by a

fusion of cells and not by a splitting apart of the cells and the development of secreting cells about this intercellular space. In his earlier paper Brebner states that the mucilage ducts are schizogenous, but in his later paper he admits that they may be of lysigenous origin, which certainly is the case in the species of *Danæa* described here.

The details of the older central bundles of the sections *F* and *G*, fig. 150, are shown in fig. 153, *E*, *F*; the shaded cells in *C* are tannin cells.

Figure 151 shows longitudinal sections of a young sporophyte of *D. elliptica*, of about the same age as the sporophyte which has just been described. This also shows three fully developed leaves, while the fourth and fifth leaves are well advanced.
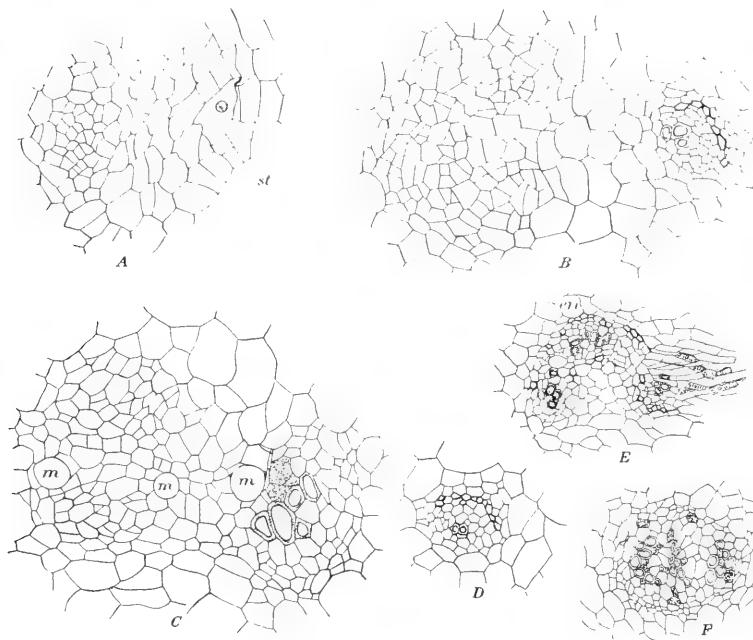


FIG. 153.—Details of the vascular system of sporophyte shown in fig. 150.
A. Passes through the stem apex; B and C, lower down, showing the fourth and fifth leaf traces. ×150.
D. A single leaf trace. ×150. E, F. Vascular bundles from the lower part of the stem. *m, m,* young mucilage ducts.

The central bundle of the stem formed by the junction of the three first leaf traces is now conspicuous and forms a stout central strand, continuing downward into the primary root $(r^1)$. *A* is a nearly median section, passing through the stem apex, whose large and conspicuous apical cell is shown in fig. 152, *B*. The apex of the fifth leaf is seen just above this and a more enlarged figure of this is shown in fig. 152, *A*. The apex of the fifth leaf (which is here cut transversely) is occupied by a small group of large cells, of which the central one may perhaps represent a single initial cell. Below the stem apex, but separated from it by a considerable amount of tissue, can be seen the bases of the leaf traces from the fourth and fifth leaves respectively. These are joining the central bundle of the stem near the junction

of the third leaf trace, and at this point there may be seen the base of the third root ($r'$). Between the fourth and fifth leaf traces is present a section of the first mucilage duct. $B$ is a section some distance to one side of the apex, also passing through the primary mucilage canal and the fourth root, which arises between the fourth and fifth leaf traces. Figures $C$ and $D$ are sections on opposite sides of the stem apex from $B$. The fourth leaf, with its conspicuous stipules ($st$), shows in these sections, and the third root can also be seen. Fig. 152, $C$, is a more enlarged view of the third root shown in fig. 151, $D$. The root is triarch and in the peripheral part of the cortex there is visible a row of very conspicuous cells whose walls stain very strongly with safranine and in section closely resemble large tracheary elements. A longitudinal section of these cells, which are also conspicuous in the later roots (fig. 155), shows that they are elongated sclerenchyma cells whose thick walls are conspicuously pitted. Farmer (Farmer 3) observed similar cells in the roots of *Angiopteris*.
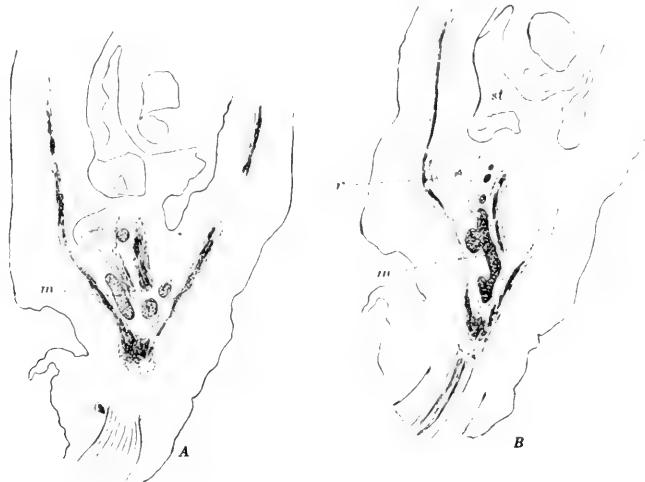


FIG. 154.

Two sections of an older sporophyte of *D. jenmani*. ×18. *m*, mucilage ducts; *st*, stipules of young leaf; *r*, young root.

Figures 154 and 155 show three longitudinal sections of a pretty well advanced young sporophyte of *D. jenmani*. The root (shown in the figure emerging at the base) is probably the second root. The stem has already begun to assume a dorsi-ventral form, and the basal part of the root, which is strongly curved, is cut away and so does not show in these sections. The earliest leaves are no longer recognizable and it is impossible to determine just how many leaves have been formed. The leaves first formed have the single trace characteristic of the first leaves in all of the species, but the youngest leaves show that the leaf trace is double. In this section (fig. 155, $A$) is shown a very young leaf, cut through parallel to its surface and showing clearly the stipules at its base and also the young vascular bundles. Within the leaf are two bundles, which are seen to join at its base, but separate again lower down, the two bundles thus forming two distinct traces in the stem. In fig. 154, $B$, the section passes through the youngest root, which is seen to have its stele joined to one of the leaf traces from the youngest leaf. Several conspicuous mucilage ducts are now present in the stem near the vascular bundles; these are

formed of rows of large cells which become more or less fused together and their contents, composed of a dense granular mucilage, stain very strongly, either with safranine or with Bismarck brown, which were the principal stains used in preparing the sections.    Numerous tannin cells are also present, scattered irregularly through the cortical tissue and also in the vascular bundles.

Figure 155, *B*, is a median section through the base of one of the later roots, showing its connection with the corresponding bundle in the stem.   The elongated cells (*sc*) are the cortical sclerenchyma cells already referred to.   These are shown on a larger scale in *C* and *D*, where the conspicuous pitted walls can be seen. The shaded cells in the figure are tannin cells.

Figure 156 shows cross-sections taken at different heights from a sporophyte of *D. jenmani*, a plant of about the same age as that which was shown in the preceding series of longitudinal sections.   Sections made near the apical region, *A*, *B*, and *C*,
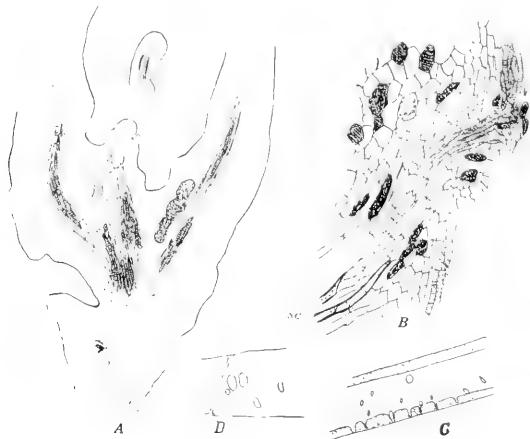


FIG. 155.

A. Another section of specimen shown in fig. 154.
B. Base of a root showing sclerenchyma, *sc*, in cortex.   ×40.
C. Sclerenchyma cells, showing pitted walls.   ×320.
D. Surface view, showing pits.

show two pretty well advanced leaves and the first trace of a third, the apex of which is indicated in *C* and *E*.   Unlike the earlier leaves with their single leaf trace, these later leaves, as we have seen, have the petiole traversed by two vascular bundles whose sections are plainly visible.   These, as we have remarked from a study of the longitudinal sections, fuse into one near the base of the leaf (*A*), and two small bundles are also present on either side, belonging to the stipules.   As the sections are followed downward, the main bundle is found to divide into the double leaf trace entering the stem, and the two small stipule bundles become united with these, so that within the stem, at about the level of the stem apex, two distinct bundles are seen, corresponding to each leaf.   The leaves are now arranged in two series, indicating that the dorsiventral character of the stem in this species is established and the youngest leaf lies almost opposite the next youngest one, whose leaf traces can be seen.   *E* shows the stem apex with the apical cell, *x*, and the youngest leaf rudiment, *l*.

Below the level of the stem apex, the stele of the axis closely resembles that already described from the somewhat younger plant and forms a somewhat irregular crescent, evidently composed of two portions which are joined on the side facing the two leaf traces from the oldest leaf which shows in this section. The opening of the crescent at this level is turned away from the leaf. No permanent elements are present in the central stele as yet. Following this downward, the crescent is seen to open on the side next the two oldest leaf traces and there is thus formed a foliar gap, while on the opposite side the space becomes closed. In section *D*, which is taken lower down, the position of the section of the central stele is reversed. At



FIG. 156.

A-D. Four transverse sections of a young sporophyte of *D. jenmani*.  ×20.  Section C shows stem apex.
   E. The apical meristem with apical cell of stem, *x*, and a leaf initial, *l*.  ×180.
F-I. Four sections from the same series, but taken lower down.  ×20.  Figs. F, G, show fusion of double leaf trace with central stele.  *m*, mucilage ducts.  Between the mucilage ducts in G can be seen the small commissural strand.

this point the section of the central stele forms a crescent of tissue, most of which has not passed beyond the procambial stage. This crescent-shaped mass is evidently composed of two parts and represents mainly the basal undivided bundles of the two youngest leaves. Tracheary tissue has begun to develop in the older of these two masses. Occupying the space within this crescentic stele are three young mucilage canals, but none are yet developed in the cortical tissue of the stem. Within this central space there can also be seen the section of a small isolated group

of procambial cells. This is the single axial stele or commissural strand which had not yet begun to develop in the plant with five leaves. The older leaf base, shown in this section, has a single large vascular bundle, crescent-shaped in outline and concentric in structure, and there are also seen the two small concentric bundles belonging to the stipules.

All the leaf traces in the older leaves are concentric in structure, with well-developed internal phloem, and thus differ from the collateral traces of the earliest leaves. This concentric structure of the bundle is already well marked in the fourth leaf, where, however, the protophloem is mainly developed upon the outer side. *F* shows the arrangement of the bundles in a section somewhat further down. The two strands from the oldest of the three leaves seen in the apex have joined the horns of the crescentic central bundle, which still shows clearly its dual nature. In the younger of the two portions of these sections the first tracheids have just appeared near the free end, while in the older half a line of tracheids extends nearly through the whole of the center. The mucilage ducts at this level are much larger and their cells have become confluent. Close to the large mucilage canal can be seen the
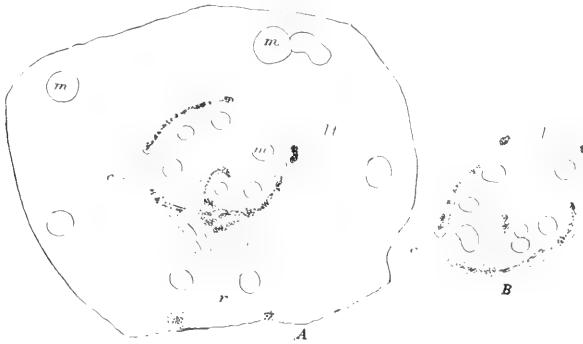


FIG. 157.

A. Section of an older stem of *Danæa jenmani*, showing cortical mucilage ducts, *m*, and central or commissural strand, *c*. *l t*, leaf trace. *r*, root. ×20.
B. Shows a section higher up, with free leaf traces and commissural strand.

section of the axial vascular strand, which somewhat higher up turns outward and becomes fused with the inner face of the crescentic central stele. The leaf traces soon become completely merged with the broad crescentic stele and their limits can no longer be recognized. The foliar gap gradually closes up and the two portions of the crescent separate at a point opposite so as to form a leaf gap, nearly opposite the first one; but I have seen no cases where a section of the central stele appears as an unbroken ring, although it is possible that this might occur for a brief period. Much the same condition of things is seen lower down and it is clear that in all cases, after the double leaf trace has been developed, one bundle fuses with an older and one with a younger leaf trace. The axial bundle shows a central mass of tracheary tissue, and in somewhat older stages an endodermis is pretty well developed. Finally this bundle can be traced downward to where it connects with the main stele, composed of the united leaf traces. The main stele gradually passes down into the base of the stem, where it shows the structure already described for the younger sporophyte.

In older sporophytes the crescentic central stele becomes broken up permanently into two elongated masses when seen in section (fig. 157), but the junction of the

leaf traces with these takes place exactly as in the younger plant. Fig. 157, *B*, shows the separate leaf traces and the free axial strand, while in *A* the leaf traces are becoming fused with the broad central strands and the axial strand is also anastomosing with one of the latter. This has taken place at the point of junction with a root. While in the younger sporophyte the mucilage ducts are confined to the central region, in the older plant they occur also in the peripheral region of the stem (fig. 157, *m*). The details of the vascular bundles are shown in figs. 158 and 159.
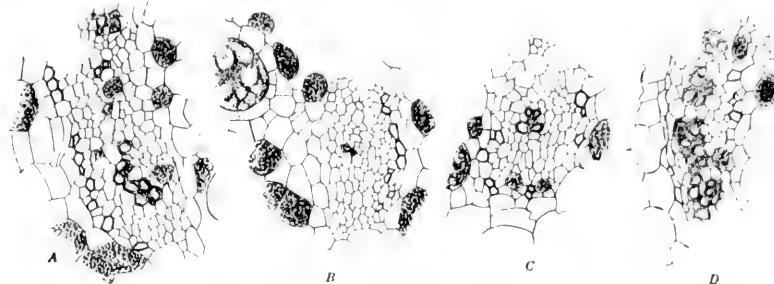


FIG. 158. – Details of vascular bundles from sections shown in fig. 156.

A and B are the ends of the crescentic bundle shown in fig. 156, D ; C, is a leaf trace and D part of an older bundle.    × 150.

To recapitulate: The vascular system in the young sporophyte of *Danæa* begins as a single axial strand, which is continuous through the cotyledon and root. At a very early period a second vascular bundle or stele is formed in the second leaf connecting with the primary strand, and this is followed by a similar single strand or stele in each succeeding leaf, up to about the seventh. Up to this time, except for the steles of the secondary roots, the whole vascular system is built up of united
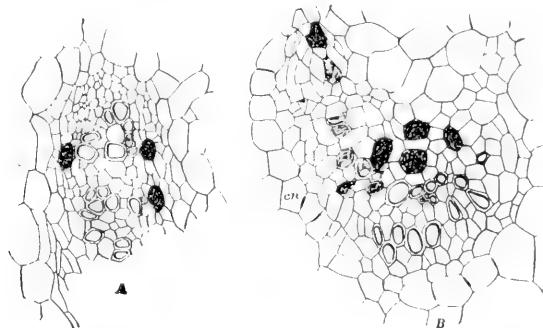


FIG. 159.

A, part of central stele; B, the commissural bundle from a young sporophyte of *Danæa jenmani*.  × 150.

leaf traces and there is no cauline bundle in the strict sense of the word, although we may speak of the bundle, or stele of the stem, as soon as there is a solid central strand formed below the junction of the earlier leaf traces. This primary stele never has the form of a true protostele, however, as the xylems belonging to the separate leaf traces can be recognized and the compound nature of this central bundle is unmistakable.

At a later stage, probably about the time that the seventh leaf is formed, there arises the single axial (commissural) strand, which is cauline in its origin and which, as Brebner has stated for *D. simplicifolia*, is the only strictly cauline portion of the vascular system of the stem. This can first be seen at about the same time that the first double leaf trace appears.

The development of the vascular system was not traced further, as it has already been adequately described by Kühn (Kühn **2**), and except for the increased number of leaf trace and commissural strands the vascular system is essentially the same in a young plant like that shown in fig. 157 as it is in the adult sporophyte.

Brebner's account of the development of the young sporophyte in *Danæa simplicifolia* agrees essentially with my own studies of *D. elliptica*, *D. jenmani*, and *D. jamaicensis*. He failed, however, to get successful sections of the transition region between the root and the cotyledon, or, as he puts it, "between the root and stem," and his statements that the central bundle begins as a "haplostele," i. e., a protostele, is not borne out by my studies on the other species. Moreover, his statement that "in this way the somewhat irregular adelosiphonic dictyostele of *Danæa* is established now by a process which is, to all intents and purposes, a branching of the haplostele, due to the departure of the leaf traces of closely set, spirally arranged leaves," might better be stated, "the dictyostele arises by a process which is essentially the fusion of the leaf traces of closely set, not necessarily spirally arranged leaves." He fully recognized the important part which the leaf traces play in the building up of the dictyostele of the stem, but he did not apparently recognize these as the sole factors in the development of the dictyostele of the older stem. He does, however, specifically state that the axial or commissural strand is the only part of the vascular system which is strictly of cauline origin.

## THE ADULT SPOROPHYTE OF DANÆA.

The anatomy of what was supposed to be the sporophyte of *Danæa* was first described by Holle (Holle **2**), but the later investigations of Kühn showed that the plant investigated by Holle could not have been a *Danæa*, nor could it even have belonged to the Marattiaceæ, since Holle describes the rhizome as developing a sheath of sclerenchyma, a condition of things which has not been found to exist in any Marattiaceæ. Kühn's account is very far from complete and the description of the sporophyte given by Brebner (Brebner **3**) for *D. simplicifolia* does not deal with the adult sporophyte. The account given here is based mainly upon a study of *D. jamaicensis* and *D. elliptica*, but an investigation of certain points was also made in *D. jenmani*. The rhizome in *D. jamaicensis* and *D. jenmani* is a markedly dorsiventral one. These two species are a good deal alike, but are readily distinguished by the difference in the form of the leaves. In the former the leaves are rather larger and have more numerous pinnæ, while the terminal pinna is developed. *D. jenmani* has somewhat smaller leaves with from seven to nine pairs of leaflets, and the leaves are abruptly pinnate, the terminal pinna not being developed (plate 9, A, fig. 1).

The leaves are arranged very much as they are in *Helminthostachys* or in *Ophioglossum pendulum*, the fleshy leaf base being provided with two very conspicuous stipules which are developed very early in the life of the sporophyte. The exact number of roots corresponding to a leaf is not easy to make out, but there are at least two, and it is quite possible that the number may sometimes be greater. These roots branch freely, especially toward the tips.

*D. elliptica* (plate 10) is a good deal larger than the other species and the stout rhizome, which, including the persistent leaf bases, has a diameter of 3 centimeters

or more, is upright, the leaves being spirally arranged as they are in *Marattia* and
*Angiopteris.* The petioles in this species are characterized by curious enlargements
or nodes, which may occur in the other species also, but are much less prominent.
The leaves of *D. elliptica* reach a length of about 50 centimeters and the leaflets,
which are much larger than those in the other species, are less numerous, there being
usually about eleven. The conspicuous nodose swellings seen in the petiole are
repeated on a smaller scale in the rachis at the base of each pair of leaflets. Sections
of the stems were examined in *D. jamaicensis* and *D. elliptica*, which, except for a
difference in size, agree closely in their structure. The ground tissue, as was first
pointed out by Kuhn, consists entirely of parenchyma, through which are scattered
many conspicuous tannin sacs which, in preserved material, appear to the naked eye
as numerous black specks. The section of the vascular cylinder looks very much
like that of the younger sporophytes already described, but the number of bundles
seen in the section is larger, and there is a group of several bundles instead of the
single medullary strand seen in the section of the younger stem. In a section from
a large specimen of *D. elliptica* in which the rhizome, including the old leaf bases,
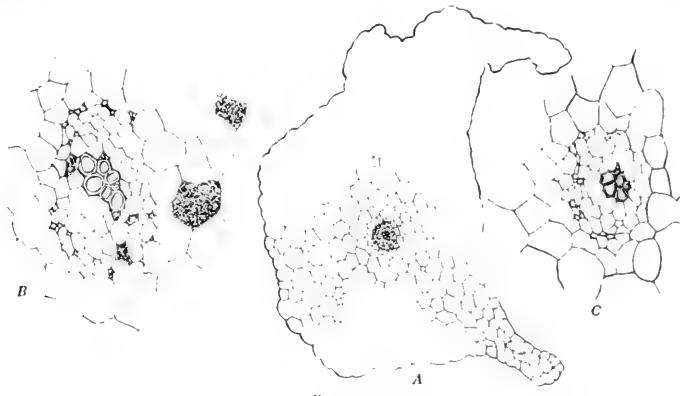


FIG. 160.

A. Section of petiole of fourth leaf of *Danæa jamaicensis*.  × 40.
B. Leaf trace.
C. Bundle of petiole.  × 180.

was about 3 centimeters in diameter, the outer ring of bundles contained thirteen,
while within this was a smaller circle composed of five medullary bundles, three
large ones and two smaller ones. In a similar section of a medium-sized section of
*D. jamaicensis*, in which the rhizome was about half the size of that in *D. elliptica*,
there were nine bundles in the outer ring and four in the middle, two large ones and
two small ones. In this section the junction of a root was cut through and the root
stele was seen to be applied by its broad base to one of the larger of the medullary
bundles (fig. 161, *A*).

The relation of this central group of bundles in the stem to the primary medullary
strand was not investigated. Brebner has shown that in the later stages of the young
sporophyte in *D. simplicifolia* the original medullary strand—which, as we have seen,
is really the only part of the vascular system which is strictly of cauline origin—
fuses at certain points with the bundles of the outer ring, and at this point of fusion
there may be a branching so that a section above this shows two of these medullary
bundles. Whether all of the central group of bundles seen in the stem of the adult

sporophyte in *D. jamaicensis* and *D. elliptica* are due to further branching of the primary medullary strand, or whether some of them may be new bundles of cauline origin, remains to be investigated.

The arrangement of the characteristic mucilage ducts is the same in the two species studied. There is a ring of these in the outer region of the cortex and a second group in the central region, these central ones usually being close to the vascular bundles.
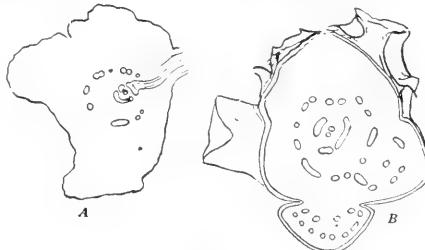


FIG. 161.
A. Section of adult rhizome of *Danæa jamaicensis*. ×2.25
B. Section of rhizome of *D. elliptica*. ×1.5.
The mucilage ducts are not shown.

## THE ANATOMY OF THE LEAF.

A section of the petiole of the adult leaf appears very much like a similar section of the stem, except that there is developed in the cortical region, separated by several rows of cells from the epidermis, a band of sclerenchyma, yellowish or brownish in color, and very much indeed like that common in the typical ferns. Holle's statement that a similar mantle of sclerenchyma occurs in the stem was shown to be an error, the result of his having investigated a fern which was not a *Danæa*. There certainly was no trace of sclerenchyma in any of the specimens I have examined.

The distribution of the vascular bundles in the petiole is very similar to that in *Helminthostachys*, except that inside the ring of bundles there is a single larger central one, instead of the two small ones which occur in *Helminthostachys*. All the bundles are continued into the stem as separate strands or there may be a certain amount of anastomosing among them, as there is in *Ophioglossum pendulum*, which



FIG. 162.—Apex of small root of *Danæa jenmani*. ×200.

*Danæa* resembles more in the character of its leaf traces than it does *Helminthostachys*, where the bundles of the petiole are united into a single trace within the cortex of the stem.

The distribution of the mucilage ducts in the petiole is very much like that of the stem. There is a definite ring of these inside the zone of sclerenchyma and several larger ones in the central region, near the vascular bundles. The form and venation of the leaf is essentially the same as in the younger sporophytes which have already been described, and stomata are developed only upon the lower side, the epidermal cells showing a strongly undulate outline, except for the series of narrow cells which

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immediately surround the stoma. A section of the lamina presents an appearance very much like that of *Helminthostachys*. Below the upper epidermis is a well-developed palisade layer, while below this the spongy mesophyll has the characteristic intercellular spaces found in the leaves of most of the higher plants. *Danæa tricho-manoides*, a very small species from Peru, has delicate membranaceous leaves, which probably would show a much simpler structure than that of the coriaceous leaves of the larger species. *D. sintensis*, a species from Porto Rico, which is in the herbarium of the British Museum, has adventitious buds developed at the leaf tip.



FIG. 163.

A, B. Two longitudinal sections of a young sporophyte of *Kaulfussia*. $l^2, l^3$, second
and third leaves; $r^2$, second root. ×20.
C. Stem apex. ×180. D. Apex of third leaf. ×180.

### THE APICAL GROWTH OF THE ROOTS.

The earlier roots grow from a single initial cell, but this is later replaced by a group of similar initials which, in the large roots, are very much like those described for the root apex of *Angiopteris* (Koch 1). Figure 162 shows a section of a root from the young sporophyte which is somewhat transitional in character between the form with a single definite initial cell and the larger root with its group of initials. The cell *x*, which is very much like the apical cell of the primary root, may perhaps still be considered as the single apical cell, but the adjacent segments are almost equal in size to this, and it is clear that they contribute to the plerome cylinder of the root as well as to the cortex and the root cap.

In sections of large roots taken from the adult sporophyte, there is found at the growing point not a single initial cell, but a group of apparently similar initial cells somewhat wedge-shaped in longitudinal section and arranged in a radiating fashion. No single one of these can be clearly recognized as a primary initial cell. These cells divide at intervals by longitudinal walls and from the bases additions are made

to the plerome cylinder, while from the outer cells of the meristem group arise the new cells for the root cap. The outer segments cut off from the peripheral cells of the apical group of cells undergo more rapid periclinal divisions than those nearer the center and the cells thus formed contribute to the cortex of the root.

Unlike the roots of most of the Ophioglossaceæ, those of *Danæa* branch freely in a monopodial fashion. This is paralleled among the Ophioglossaceæ by some of the larger species of *Botrychium*, *Helminthostachys*, and *Ophioglossum pendulum*. Kühn's statement that the roots are unbranched is incorrect. The branching of the roots was perhaps somewhat more frequent in *D. jamaicensis* than in *D. elliptica*, but the latter species also shows numerous short lateral rootlets. The root hairs are not conspicuous on these older roots, but are readily seen upon the younger ones and are then found to be multicellular, as was first shown by Brebner for *D. simplicifolia*. Sections of the larger roots were examined in these two species and, while they agreed in the main, there were some slight differences. In a large root of *D. elliptica*, about 2 millimeters in diameter, the outer portion was occupied by about four rows of brownish thin-walled cells, very probably more or less suberized. Within this was a ring of brownish sclerenchyma cells about three cells in thickness, and between this and the exceedingly conspicuous endodermis was a broad zone of parenchyma, the cells containing much starch. A short distance outside of the endodermis was a ring of conspicuous mucilage ducts. There were twelve xylem rays alternating with as many rays of phloem. These did not extend to the middle of the stele, which was occupied by a pith of considerable size. The roots of *D. jamaicensis* were somewhat smaller and the main differences were: first, the thicker layer of tissue outside the ring of sclerenchyma, which was much less developed than in *D. elliptica* (in many places this ring was but one cell in thickness and very seldom more than two cells); second, the mucilage ducts were relatively very much larger; third, there were but nine xylem masses instead of the twelve in the root of *D. elliptica*.

The endophyte, which is usually found in the primary roots of all the Marattiaceæ is generally wanting from the larger roots of the adult sporophyte.

## THE SPOROPHYTE OF KAULFUSSIA.

The development of the young sporophyte in *Kaulfussia* resembles that of *Danæa* in most respects, but from the first it is markedly dorsiventral, like the sporophyte of *Helminthostachys*. The cotyledon, while not provided with the characteristic free stipules of the later leaves, still has its base connected with a ridge which extends around the stem apex and youngest leaves, which thus lie in a cavity at the base of the cotyledon in much the same way that the stem apex is situated in the young sporophyte of *Ophioglossum;* but the sheath in *Kaulfussia* forms a shallow open cup, instead of the closed conical sheath found in *Ophioglossum*. This stipular sheath seems to be in all respects similar in its origin to that found in *Ophioglossum*.

The second leaf lies nearly opposite the first and the third next to the first, and so on, the distichous arrangement being evident from the beginning. We have already pointed out that in the very young sporophyte the vascular bundle from the cotyledon is continued directly into that of the root and that the bundle from the second leaf joins this common bundle, as shown in fig. 163. These primary bundles run on one side of the apical meristem, which, as in *Danæa*, is of very limited extent and does not contribute at all to these bundles.

The apical cell of the young sporophyte, up to the oldest stages that were investigated, is very much like that of the very young plant and is broader than that of *Danæa*, more resembling both in cross and longitudinal sections the apical cell

of *Ophioglossum*. The young leaves arise right and left in succession on either side of the stem apex, and from a very early period are strongly bent over, showing the characteristic circinate vernation of the Marattiaceæ. Sections made through the apex of the young leaf show that it has at first a definite apical cell a good deal like that which is found in the stem apex, and although no satisfactory cross-sections of this apical cell were found, there seems to be no question that there is present a single definite initial cell which probably has much the same form as that of the stem apex. The young leaf, at this stage, has the dorsal region strongly convex and composed of large parenchyma cells. The apex is bent over forward and downward.
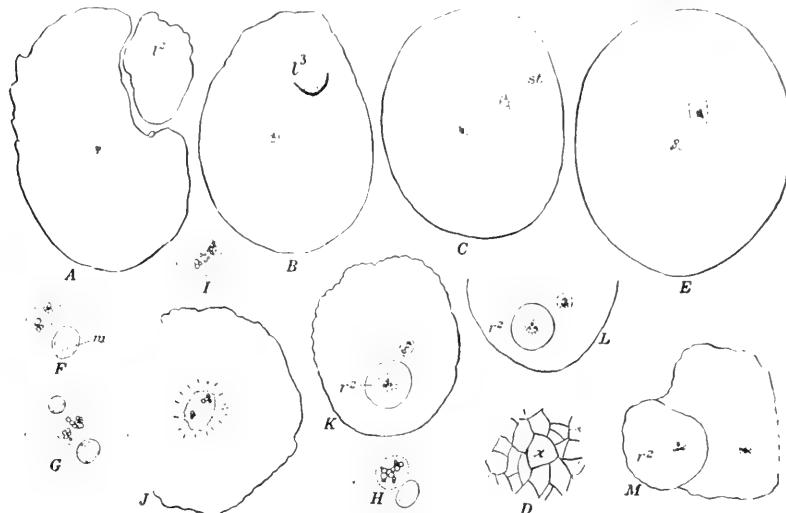


Fig. 164. Series of transverse sections from a young sporophyte of *Kaulfussia*, showing the coalescence of the two primary leaf traces. $l^2$, second leaf; $l^3$, third leaf; $r^2$, second root. ×40.
   D. Stem apex more enlarged.
   F–H. Central region of the sections. The fusion is complete in H. The shaded area is a mucilage duct.

The tissues of the apex and the adjacent region are composed of small, actively dividing cells. The young vascular bundle can be clearly seen extending nearly to the apex and continuing downward into the stem until it joins the bundle from the next older leaf.

The second root arises at about the same time that the third leaf is recognizable and apparently its origin is exactly the same as it is in *Danæa*. Its stele joins the central bundle of the stem near its junction with the third leaf trace. The young leaves, like those in *Danæa*, are sparingly covered with hairs and scales, but these scales do not have the peltate form found in *Danæa*, being usually short rows of stout cells; or the hair may have its base composed of an oblong mass of cells attached by a narrow stalk and having its apex drawn out into a slender filament composed of two or three elongated cells. The terminal cells of these hairs usually stain very strongly, indicating that they contain tannin.

The young leaves are relatively stouter than in *Danæa*, but otherwise resemble them closely. In the young sporophyte, up to the time of the formation of the third leaf, there may be no trace of the mucilage ducts, although these may be developed

at an earlier period. The mucilage ducts throughout the life of the sporophyte are less conspicuous in *Kaulfussia* than in *Danæa*.

A section taken at the level of the stem apex in the very young sporophyte (fig. 137) presents an appearance not unlike that found in the bud in *Ophioglossum*. The base of the cotyledon extends around the stem apex, so that in the section the stem apex and the second leaf are inclosed in this cavity formed by the stipular sheath of the cotyledon. Higher up, the base of the cotyledon becomes free from the second leaf, but this takes place earlier on one side than on the other.

The development of the vascular system in the stem is exactly as it is in *Danæa*. The stem apex in the young sporophyte takes no part in the development of the vascular bundles, the stele in the stem being made up entirely of the united leaf traces. In the specimen figured (fig. 164), in which the second leaf was still quite young, the bundle from the cotyledon passes downward into the stem and continues its downward course until it joins the second leaf trace. The resulting solid stele at first shows the two separated xylems of the component leaf traces which lower down merge into a single thick band of xylem completely surrounded by the phloem



Fig. 165.—Three longitudinal sections of a young sporophyte of *Kaulfussia*, with three leaves. ×25.

(fig. 164, *E–H*). This stage corresponds to Brebner's "haplostele" in *Danæa simplicifolia*. This condition merges insensibly into the primary root with its diarch bundle, the two xylems in the younger part of the root being quite separate, but forming a single band at the base of the transitional region, between the root and the bundle in the stem. The bundle of the root shows a conspicuous endodermis which becomes less and less evident in the transitional region, although it probably never quite disappears. The primary root usually shows the presence of an endophytic fungus like that occurring in the prothallium and in the primary root of most of the Ophioglossaceæ.

Fig. 166 shows cross-sections of a young sporophyte in which the fifth leaf is still quite small, but with its trace showing the first tracheary tissue. This fifth leaf trace unites below with the trace from the fourth leaf and forms a single bundle, with the xylems separate. The xylem from the fourth leaf above its junction with the fifth leaf trace forms at first a continuous band which divides into two parts, one of which unites with the xylem from the fifth leaf trace, while the other remains distinct. This separation of the xylem in the fourth leaf trace is the first indication of the formation of the double leaf trace, such as we shall find occurs in the later leaves.

The separated xylems become joined lower down into a single transverse plate, just
before the junction of the strand with the trace from the third leaf (fig. 166, *II*),
with which it joins in the same way and is continued downward to the junction with
the strand arising from the fusion of the second leaf trace with the cotyledon. Above
the junction of the first and second leaves, the stele of the second root, which grows
vertically downward, joins the stele formed by the junction of the three youngest
leaf traces.

Above the junction the section of the bundle is nearly circular in outline, the
large tracheids forming a broken group in the center of the bundle, quite like a



FIG. 166.

A–C. Series of transverse sections from a sporophyte of *Kaulfussia*, of about same age as that shown in fig. 165.
D. Stem apex.
H–J. Three sections lower down.
K. Central vascular cylinder still lower down.

corresponding stage in *Danæa*. Some of the cells adjacent to the bundle show the
typical thickenings on the radial walls, indicating that they belong to the endodermis,
but the limits of the endodermis are extremely vague. In this plant two mucilage
ducts had developed in the fourth leaf, one on each side of the vascular bundle
(fig. 166, *B*, *m*).

The second root is stouter than the primary one and its bundle is triarch. The
mycorrhiza present in the first root could not be detected in the second root, but
possibly may be developed at a later stage. All of the roots are provided with root

hairs, which resemble those in *Danæa* in being multicellular. In the larger roots there may sometimes be five or six cells, each with a conspicuous nucleus, making up these root hairs, and sometimes they show signs of branching at the apex.

It is evident that in *Kaulfussia*, up to the time that five leaves have developed, the vascular system of the stem consists of a single axial strand, a sort of sympodium formed by the completely united traces of the young leaves. The xylem may appear as a single mass at the points where fusion of the leaf traces is complete, but at most points the individuality of the component strands is maintained, so far as the xylem is concerned.

Figure 168 shows transverse sections from a much older sporophyte. The section of the petiole still shows the single vascular bundle having near its inner face a single large mucilage duct, which follows the leaf trace downward into the stem. As the bundle from the leaf enters the stem it broadens, and there is a separation of the xylems into two masses, first seen in the fourth leaf. In the specimen shown here the earliest leaves were not sectioned, and, as it can not be stated exactly how many leaves the young sporophyte had developed, it must therefore remain uncertain which leaf first shows the completely divided leaf trace. In the specimen in question the oldest leaf that showed had a single trace with two separate xylems,
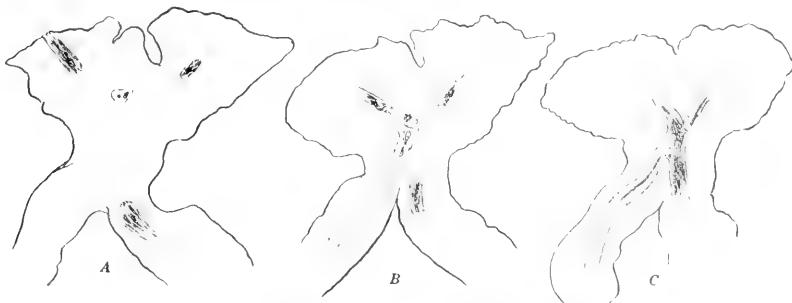


FIG. 167.—Three longitudinal sections of an older sporophyte of *Kaulfussia*. ×25.

while the next leaf had the leaf trace completely separated and resembling a corresponding stage in *Danæa* (fig. 168, *F*). As the traces from the two leaves approach preliminary to their fusion, the double leaf trace has its parts united again so that the traces become single before they join. The leaf trace now forms a single bundle, crescentic in section and very much like the single broad leaf trace from the earlier leaf. The two traces come nearer and nearer and finally fuse on one side, so that a section shows a single bundle almost circular in form, but broken on one side (fig. 168, *H*). This break is finally closed up, and the section of the bundle is completely circular and resembles very closely that of *Botrychium* or *Helminthostachys*, except that phloem is developed inside the ring of xylem (fig. 168, *K*). A similar condition may sometimes be found for a very short time in the young plant of *Danæa* also.

No certain evidence of an internal endodermis could be made out and the external endodermis is also somewhat vague, although there is probably no question of its presence. The outer phloem is bounded by a fairly well-defined pericycle, between which and the starch-filled cortical cells of the stem are two or three layers of transparent cells, some of which presumably constitute the endodermis. The endodermis is certainly present in the free leaf traces, although less conspicuous

than it is in the roots. In the upper region of the stem the leaf traces are further apart, and as the number increases a cross-section of the stem shows a circle of separate bundles closely resembling the arrangement in *Ophioglossum*. The central "commissural" strand, which is found in the adult stem, had not developed in any of the young sporophytes I examined and evidently arises much later in the history of the sporophyte than it does in the other Marattiaceæ. In this respect, as well as in the arrangement of the bundles themselves, *Kaulfussia* is more like
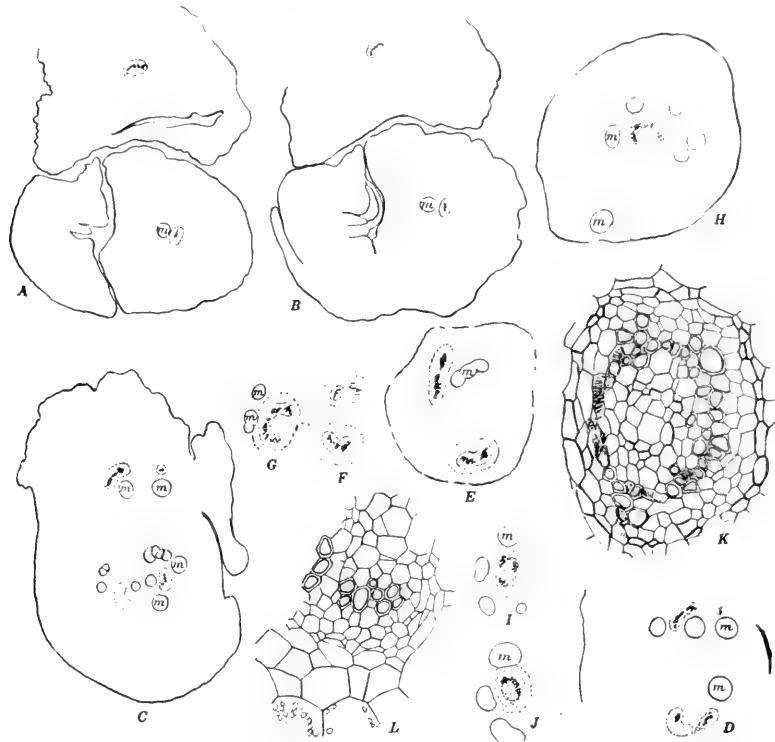


FIG. 168.

A, B. Two sections above level of stem apex of a young sporophyte of *Kaulfussia* of about same age as that shown in fig. 167.
C–J. Sections taken lower down from same series.  *m*, mucilage ducts.
K. Central bundle of section J.  ×140.
L. Part of the bundle of section H, more enlarged.  G, F, I, J, show only the central region of the sections.

*Ophioglossum* than it is like the other Marattiaceæ. In the intermediate condition between the single solid stele found in the very young stem and the entirely separated leaf traces of the older rhizome, there is a transitional condition with a "siphono-stele," very much as in *Helminthostachys* (fig. 168, *K*).

In one very young sporophyte there was found, in the cortex of the primary root near its base, a large lacuna which recalled the similar lacuna found in the first internode of *Helminthostachys*.

The young sporophyte of *Kaulfussia* is almost completely destitute of the tannin cells which are so conspicuous a feature in *Danæa*, and these tannin cells are practically entirely absent from the sporophyte throughout its whole existence. In this respect there is a marked resemblance to *Ophioglossum*. The mucilage ducts are also somewhat less developed than in the other Marattiaceæ and are mainly confined to the central part of the stem in proximity to the vascular bundles. At a late period they may also appear in the cortical region. Their lysigenous origin is less evident than in *Danæa* and it is not impossible that they may sometimes be of schizogenous origin, as Brebner states is often the case in *Danæa*.

The structure of the vascular strands is very much like that of *Danæa*, as a reference to the figures will show. There are developed slender, spirally-marked protoxylem elements like those in *Danæa* and which we have already seen occur also among the Ophioglossaceæ, in *Helminthostachys*.

The stipules in the young plants of *Kaulfussia* are much less definite than they are in *Danæa*. A cross-section through the bases of the young leaves shows that instead of the bases appearing free, each with its distinct stipules, the leaf is confluent with the next one, one side being slightly extended and free. The leaf base thus incloses the next younger leaf completely on one side, but leaves it free on the
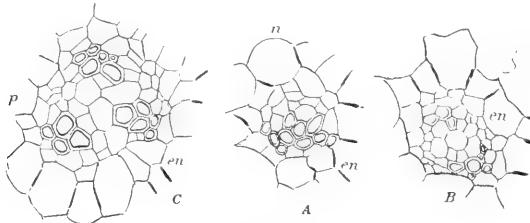


FIG. 169.

A. Bundle from intermediate region of a very young sporophyte of *Kaulfussia*.   ×150.
B. Section of primary root.   ×150.
C. Section of bundle of second root.   *en*, endodermis.   ×150.

other (fig. 168, *B*). In the later leaves the stipules become more conspicuous, but they are always connected by a very conspicuous commissure in front (fig. 171, *com*) so that a section of the young leaf base shows a space between the front of the petiole and the stipular sheath. In short, the structure is very similar to the open stipular sheath of *Botrychium virginianum* and apparently arises in much the same way.

The structure of the adult sporophyte has been carefully investigated by Kühn, who pointed out that it is the simplest of the Marattiaceæ in the structure of the stem (fig. 171, *B*). As in the young sporophyte, the stem shows in section a single circle of vascular bundles, but there is in addition a single median strand within the circle of bundles belonging to the dictyostele, and this bundle is presumably of cauline origin, like the corresponding one in *Danæa*.

The whole vascular skeleton of the stem, when removed by maceration, was found by Kühn to be a hollow cylinder with large open meshes. These are more elongated on the ventral side and from these ventral strands alone, according to Kühn, the roots are developed. In this respect *Kaulfussia* offers a certain analogy to *Helminthostachys* and perhaps to *Ophioglossum pendulum*.

As in the other Marattiaceæ, the ground tissue of the rhizome is composed of simple parenchyma. The outer layers have their walls brown in color and show the reaction of cork. Except for the presence of the central strand, a section of the

rhizome of *Kaulfussia* presents almost the exact appearance of a similar section of *Ophioglossum*.

In a rhizome having a diameter of about a centimeter twelve bundles showed in a cross-section taken through the internode, one of these being the medullary strand. The section of a petiole of a leaf taken from the same plant is shown in fig. 171, *C*, and the structure of the section closely resembles that of the rhizome. There were eight bundles arranged in a circle, within which were two medullary strands. The cortical region was composed of several rows of brownish cells, probably similar to those in the outer part of the rhizome, and within these, separated



Central bundle from a young sporophyte of *Kaulfussia*, showing two xylems.  ×150.
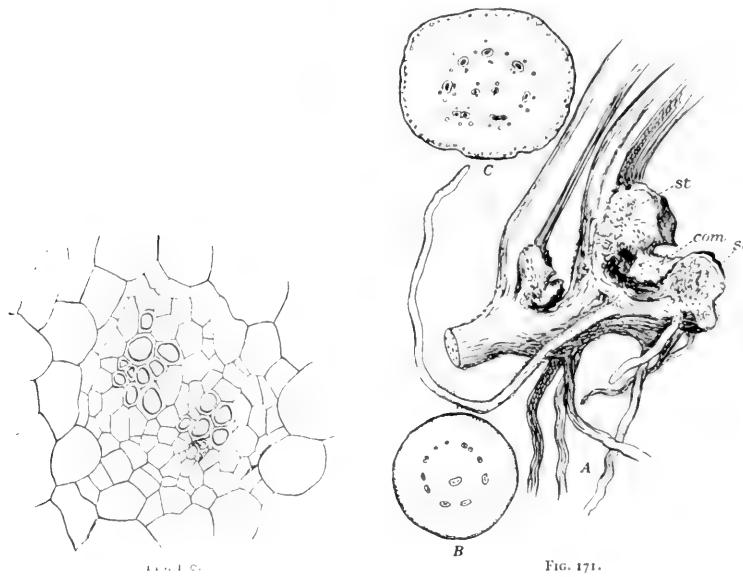
Fig. 171.

A. Rhizome of a large sporophyte of *Kaulfussia*. *st*, stipules; *com*, commissure.  ×⅔.
B. Section of rhizome.  ×2.
C. Section of petiole.  ×2.

from them by three or four rows of cells, was a conspicuous band of collenchyma, a tissue characteristic of the leaves of most of the Marattiaceæ.

According to Kühn, the bundles of the leaves are continued separately into the cortex of the stem and do not unite into a single leaf trace. There are occasional anastomoses of the vascular bundles of the petiole, quite like those in the stem itself, and this becomes especially marked at the base of the leaf, so that the leaf trace (if such it can be called) forms a hollow reticulate cone, quite equaling in diameter the vascular cylinder of the stem itself, and attached to this by the open base, the separate strands forming a crescent-shaped group when seen in section. The arrangement of the bundles within the petiole itself and their method of junction with the vascular system of the rhizome are very much like those in *Ophioglossum pendulum*.

The vascular bundles, both in the petiole and rhizome, are concentric. The endodermis is not recognizable and the central xylem is completely inclosed by the phloem, sieve tubes being developed throughout.

While mucilage ducts are present in both leaf and stem, they are less numerous and conspicuous than in *Danæa*. There is a ring of them in the outer cortex and several larger ones in the central region. Kühn states that, although these mucilage ducts sometimes look as if they were of schizogenous origin, in reality they are always lysigenous, i. e., they are formed by the fusion of several mucilage-secreting cells.

In the cotyledon and the earlier leaves a section of the lamina shows, between the large-celled epidermis of the upper and lower sides, a mesophyll composed of about three layers of quite indifferent parenchyma, exactly as it is in *Ophioglossum*. In the adult leaves the upper part of the mesophyll is compact, but there is no proper palisade tissue developed. Toward the lower surface the mesophyll becomes looser.

Until the plant has reached a considerable size the leaves remain undivided and, except for the much greater size, closely resemble the early leaves of the young sporophyte (see plate 11, fig. 1). The simple leaves are succeeded later by trifoliate ones, and finally five leaflets are developed. The long, fleshy petiole may reach a diameter of a centimeter or more and a length of some 50 centimeters in large specimens. The venation strikingly resembles that of a dicotyledonous leaf, the pinnately arranged, secondary veins being connected by a plexus of small veins, inclosing nearly square areoles within which a few free terminations are occasionally found. The large pores upon the lower side of the leaf, formed by the greatly enlarged stomata already referred to in the younger leaves, are plainly visible to the naked eye, and sections of these look curiously like the pores upon the thallus of certain Marchantiaceæ (fig. 128).

The stipules of the older leaves are very conspicuous and show much the same structure as that noted for the earlier leaves. The young leaf is included within the stipular sheath of the next older one, and near the apex the unbroken stipular sheath of the youngest expanded leaf shows very plainly that the large stipules are joined in front by a broad commissure, which extends entirely around the apex of the shoot, exactly as it does in *Helminthostachys*, from which the sheath differs mainly in the fact that it is divided above into the two stipules as it is in *Botrychium virginianum*, and there seems no reason to doubt that we have to do with entirely homologous structures (fig. 171, *A*). The stipules of the young leaves are covered with hairs and scales similar to those noted in the young sporophyte.

The roots arise from the ventral surface and flanks of the rhizome, and out-number the leaves, to which they apparently bear no definite relation.

The apical growth was not studied in the root of the older sporophyte, but it is probable that in the large roots of the older plant the single apical cell found in the early roots is replaced by a group of initials such as are found in the other Marattiaceæ, although it is barely possible that the single apical cell may be retained here as it is in the Ophioglossaceæ. Unfortunately, material was not available for a study of this point. The roots in most cases are quite unbranched, as they are in *Euophioglossum*. The only cases where branches were seen, were young sporophytes in which the end of the root had been destroyed and two branches had arisen on each side of the destroyed apex. This looks somewhat like a dichotomy and it is barely possible that, as in *Ophioglossum*, there may be a real dichotomy of the root, but all of the cases that were found had much more the appearance of the formation of two lateral roots. The young roots of the sporophyte are provided with multicel-lular root hairs, like those of *Danæa*, but these disappear as the root becomes older.

The central vascular cylinder of the root is surrounded by a circle of very large mucilage ducts. The endodermis is clearly evident and the roots which I examined

from a medium-sized plant were tetrarch. Kühn states that pentarch and hex-
arch roots also are formed. The reduced number of xylems in the root of *Kaul-
fussia*, as compared with the other Marattiaceæ, is another indication of its probable
nearer relationship to the Ophioglossaceæ, the structure of the root being very similar
indeed to that of *Helminthostachys* or *Ophioglossum pendulum*.

## THE SPOROPHYTE OF MARATTIA.

The published observations upon the young sporophyte of *Marattia* are far
from complete. Kühn (Kühn **1**) has described the stem structure in young plants
of *M. fraxinea*, but it is evident that these plants were already too far advanced
to show the early arrangement of the bundles, as the stems he described had
reached a length of nearly 2 centimeters. Farmer and Hill (Farmer **3**) have also
given some details as to the early stem structure in the same species. These young
plants, especially those described by Kühn, have the stem relatively longer than is
the case either in *M. douglasii*, which I have studied somewhat in detail, or *M.
alata*, or *M. sambucina*, which I have also examined. In all of these species the stem
of the very young sporophyte is still quite short and very soon assumes the compact
globular form with the crowded leaves that it has in the adult sporophyte. It may
be said, however, that in *M. alata* the buds which develop upon the old leaf bases
have the young stem somewhat more elongated, but not nearly so much as Kühn's
figures would indicate to be the case in *M. fraxinea*.*

In the youngest specimens examined by Kühn the cross-section of the stem
showed a ring of bundles corresponding to the leaf traces and a central medullary
strand, the whole arrangement being very similar to that which is found in the
adult rhizome of *Kaulfussia*. This stage is also very similar to the condition found
in the young sporophyte of *Danæa*.

Farmer and Hill describe the vascular skeleton of the very young plant of *M.
fraxinea* as a "siphonostele" with much larger foliar gaps than those found in
*Angiopteris*, and thus more nearly resembling *Kaulfussia* or *Danæa*. The leaf
traces are at first single, but the later leaves have double leaf traces, such as we have
described in *Danæa* and *Kaulfussia*.

The writer has already published some details in regard to the young sporo-
phyte of *Marattia douglasii* (Campbell **3, 4**), and some additional facts are here
added to those that have already been published; but as the series of specimens
available for study was not at all complete, further investigation is desirable to
complete the history of the development of the vascular system in *Marattia*. The
only material available for a study of the young sporophyte in *M. douglasii* was a
series of slides made a good many years ago from material collected on the island
of Kauai in the Hawaiian Islands. This material was supplemented by a small
number of very young plants of *M. sambucina*, collected in Java. Material of older
sporophytes of *M. alata* was collected in Jamaica in the summer of 1908. Un-
fortunately, all the preparations of *M. douglasii*, except the very youngest stages,
were longitudinal sections, so that it was difficult to follow out satisfactorily the
course of the vascular bundles in the later stages.

Longitudinal sections of a young sporophyte before the cotyledon was com-
pletely expanded are shown in fig. 133. The section was cut nearly in the plane of
the cotyledon and the bent-over apex of the latter was cut so as to show plainly the
two lobes arising from the first dichotomy of its apex. The second leaf is already
formed and differs in no essential particular from the corresponding leaf in *Kaul-*

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* Since the above was written a paper has appeared (Charles **1**) describing the vascular system of the young sporo-
phyte of *M. alata*. The stele of the very young plant is described as a protostele which passes abruptly into a solenostele.

*fussia* or *Danæa*. It was not quite certain whether a single apical cell was present at this stage, but a triangular cell which could be seen at the apex was probably the apical cell of the young leaf. The root apex of this specimen had been injured so that the form of the apical cell could not be clearly made out, but it was probably the same as we have already described for the young sporophyte. The apical cell of the stem meristem was broader in outline than that of *Danæa* and truncate below.

The vascular system at this stage, as we have already indicated earlier, consists of the common bundle of the root and the cotyledon, which is joined at a point quite close to the stem apex by the second leaf trace. The first tracheary tissue is visible at this point in the form of short, reticulately marked tracheids such as we have already seen in the young sporophyte of *Danæa*. At this stage no mucilage ducts or tannin cells had developed. About the base of the young leaves are short hairs
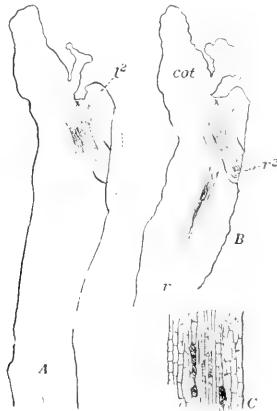


FIG. 172.

A, B. Two longitudinal sections of a young sporophyte of *Marattia douglasii*. $l^2$, second leaf; $r^2$, second root. ×140.
C. Part of primary root, showing cells infected by endophytic fungus.
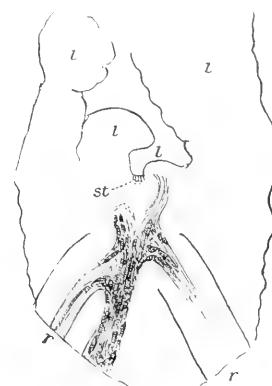
FIG. 173.

Section of a young sporophyte of *Marattia douglasii*. *st*, stem apex; *l*, young leaves; *r*, roots. ×25.

and scales like those we have seen in the other genera. They are more like those of *Kaulfussia* than like the peltate scales found in *Danæa*.

Sections of a somewhat older sporophyte are shown in figure 172, this section being made at right angles to the one just described. The cotyledon in this specimen was fully expanded and the primary root had penetrated into the earth. The arrangement of the bundles was the same as in the younger sporophyte described, except that the third leaf was now visible and the second root was already well advanced. The section passed exactly through this root, the stele of which is seen to join that of the second leaf and is practically continuous with it. The young trace from the third leaf joins the second leaf trace near its junction with the second root. The apical cell of the latter was readily seen and appears in longitudinal section of nearly triangular form, but with the base somewhat truncate (fig. 177, *C*).

The tracheary tissue in the middle of the sporophyte is pretty well advanced and the formation of the tracheary tissue has extended for some distance into the primary root and the cotyledon. In the former a single elongated tannin sac could

be seen, like those which are much more developed in the primary root of *Angiop-teris*, and it was also seen that certain of the cells in the cortex of the primary roots were invaded by the endophytic fungus. In the cotyledon there was a single large mucilage duct.

A series of transverse sections was made of a young sporophyte of *M. sam-bucina* and these agreed in all essential particulars with similar sections of *Danæa* or *Kaulfussia*.

In a young sporophyte of *M. douglasii*, in which two roots were developed in addition to the primary one, there could be seen at the apex two young leaves, prob-ably the fourth and fifth, but as only the median sections of this series had been kept the exact number of leaves could not be determined.

The central part of the stem in this plant (fig. 173) was occupied by a single thick bundle, but whether this was solid or open on one side, as Farmer states is



Fig. 174.

A, B. Two longitudinal sections of an older sporophyte of *Marattia douglasii*. *st*, stem apex; *m*, mucilage ducts. ×25. C. Stem apex. ×180.

the case in *M. fraxinea*, could not be satisfactorily determined. At this stage the section of the young plant almost exactly resembles a similar one in *Danæa*, and presumably the single central strand is composed of the confluent leaf traces from the three first leaves, as it is in *Danæa*.

The xylem of the bundle is still composed exclusively of short, reticulate tra-cheids. Large mucilage ducts and numerous tannin sacs are developed in the petioles of the older leaves and the elongated tannin sacs, like those in the roots of *Danæa* and *Angiopteris*, are sparingly developed in the young roots; but both tannin sacs and mucilage ducts were absent from the stem tissues at this period.

In a still older plant (fig. 174) the single central bundle of the basal region is replaced by the separated steles of the single leaf traces, which are beginning to form the open dictyostele characteristic of the adult sporophyte. The exact nature of its origin could not be followed in the material at my disposal, but it is presumably

much the same as that described for *Danæa,* since the appearance of the longitudinal sections in *Marattia* is exactly like that of corresponding stages in *Danæa.* The apex of the stem, however, is much broader than in *Danæa.* There is in the middle of the apical region a cell which from its size and position may be pretty certainly denominated the apical cell (fig. 174, *C*). There is present a strand of procambium which ends abruptly a short distance below the stem apex. This strand probably represents the primary commissural strand, which, as in *Danæa,* is in all probability a truly cauline bundle and has no direct connection with the leaf traces. In the central region of the stem there are now several large mucilage ducts, but tannin cells are still absent. In the roots, however, the tannin cells are abundantly developed.

Farmer and Hill's brief account of the development of *Marattia fraxinea* agrees with my own observations so far as they have gone, except for the interpretation of the vascular bundles. The "protostele" found in the lower part of the stem is undoubtedly the common bundle of the primary root and the cotyledon, and the open "siphonostele" is really made up of separate leaf traces, which anastomose at certain points to form the large meshes of the very open dictyostele. Farmer and Hill call attention to the fact that the "foliar gaps" are much wider than in *Angiopteris,* and in consequence the separate strands, seen in section, form a circle of apparently quite separate bundles, evidently closely approximating the condition found in *Danæa* and *Kaulfussia.* Farmer and Hill do not make it quite clear that the commissures which they found developed later, connecting the strands of the dictyostele, were really parts of the central cauline strand, but they presumably assume that such was the case, as this is explicitly stated in the case of *Angiopteris,* which they also studied. The structure of the bundle in the latest stage described by Farmer and Hill agrees pretty closely with the condition described by Kühn in the youngest specimens which he studied, where the stem was about a centimeter in diameter.

The early leaf traces, as in the other Marattiaceæ, are single, but later on double traces are formed. According to Farmer and Hill, the two bundles of the leaf trace unite before joining the vascular cylinder of the stem. No material was available for a further study of the development of the bundle in *M. douglasii,* but it probably agrees with that of *M. fraxinea.*

Several young plants of *M. alata* were examined for comparison with the young germ plants of *M. douglasii.* The specimens in question (fig. 175) had arisen as adventitious buds upon the old leaf bases which had become separated from the stem. This manner of formation of the young plants is very common in this species (see plate 12, A). In one of these young plants, in which the stem was about 4 centimeters long and 1.5 centimeters in diameter, exclusive of the leaf bases, the oldest expanded leaf had a petiole of about 8 millimeters in diameter. In these young plants there is usually only one leaf expanded, so that they have a very different appearance from the fully developed sporophyte with its crowded circle of leaves, and these monophyllous plants remind one very much of sterile specimens of some of the larger species of *Botrychium* (plate 12, B). A section of the petiole of this specimen showed ten vascular bundles arranged in a circle, within which, on the ventral side, were two other bundles. The section resembled almost exactly that of the petiole of a fully developed leaf of *Helminthostachys.* At the base of the leaf are the two very conspicuous stipules, one of which overlaps the other. These stipules are connected by a commissure which joins the two stipules near the base and is entirely concealed within the cavity formed by the overlapping stipules, but can be seen on raising these, as a hood-like membrane, overarching the next

younger leaf. Fig. 175, *E*, shows a young leaf from another plant, in which one side has been cut away so as to show the relation of the stipules to the leaf base. This leaf was still coiled up and its apex was quite concealed within the large overlapping stipules. Both as to its position and its relation to the stipules, the commissure exactly resembles the lip-like basal extension of the stipular sheath in *Botrychium* and *Helminthostachys*, and there is no reason to suppose that it is not exactly homologous with this.

Partially inclosed by the stipular sheath of the expanded leaf is the next younger leaf, which is cut in a plane nearly at right angles with that of the oldest leaf, so that the overlapping of the stipules, which entirely conceal the rest of the leaf, is very plainly seen (fig. 175, *B*, *F*). Within the stipular sheath of this leaf is a still younger one, which is entirely concealed from view from the outside, but shows plainly in longitudinal sections, taken next to the center of the bud. The stem apex is entirely concealed within the sheath of the younger leaf. The resemblance of this section of
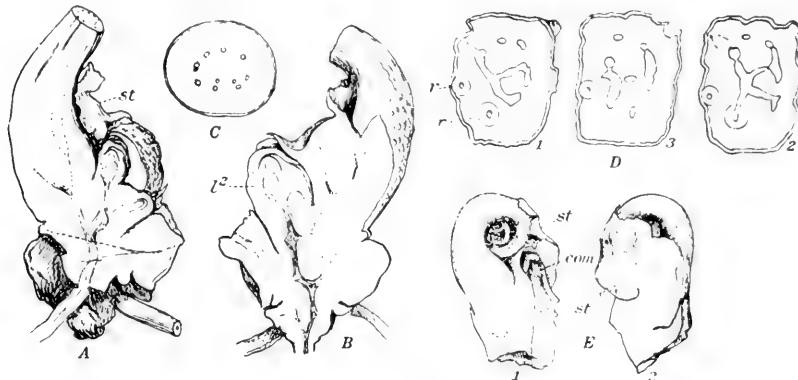


Fig. 175.—*Marattia alata* Smith.

A, B. Young plant developed as a bud upon an old leaf base. The outer tissue has been cut away, and the two sides of the thick central section are shown. ×1.
C. Section of petiole of leaf.
D. Three sections of a rhizome of a small plant. ×1.33
E. Young leaf. The lamina is coiled up within the large stipules, *st*. In (1) one of the stipules has been cut away to show the commissure, *com*. ×1.33

the young plant to a similar section of the bud in one of the larger species of *Botrychium* or of *Helminthostachys* is sufficiently striking.

The bundles forming the ring in the petiole anastomose freely in the leaf base, as they do in *Kaulfussia* or *Ophioglossum pendulum*, so that the number of strands in the leaf trace is smaller than the number of bundles within the petiole.

The vascular bundles in the stem form a single very open mesh-work, with which the leaf traces join and which is, with little question, composed entirely of these leaf traces, as it is in the earlier stage. Indeed, except for the larger size of these plants, there is no essential difference between them and the young germ plants, described in *M. douglasii*. Several roots are usually developed before the first leaf of these young bud-plants unfolds. Probably one root develops for each leaf, but it is not certain that this is always the case. Owing to the thickness of the cortical region which has to be traversed by the root before it emerges, the roots have already reached a large size before they appear upon the outside. This deep-

seated origin of the roots of the Marattiaceæ has often been figured and described. The roots from the base of this plant showed the hexarch structure, the six xylem masses being united in the middle of the stele. About half-way between the stele and the epidermis of the root is a circle of large mucilage ducts, but these were quite absent from the outer cortex. No mycorrhiza could be found, except that in the outermost layers of cells, which constitute a rudimentary periderm, occasional fungus filaments can be detected, but these are very different in appearance from the typical mycorrhiza found in the primary root of the germ plant. No root hairs could be found, but occasionally short stumps were seen which looked as if a root hair had been broken off.

Fig. 175, *D*, shows three consecutive free-hand sections of a stem from a plant of about the same size as the one we have just described, but evidently much older, as there were the remains of many leaves and the whole caudex, except for its smaller size, was very much like that of the adult plant. The broad vascular strands made up of the confluent leaf traces formed an irregular circle which in fig. 175, *D*, 2, 3, shows the free central strand which lower down forms the commissure across the central parenchyma. The arrangement of the bundles, therefore, in the young plant of Marattiaceæ is the same as in the adult rhizome of *Kaulfussia*. In these sections two roots are shown, one of which is cut through the point of junction with a strand of the dictyostele (fig. 175, *D*, 1). As in the younger germ plants, tannin cells are quite absent from the stem, although in the roots they are conspicuous. A few large mucilage ducts, however, occur in the ground tissue of the stem.

Farmer and Hill give one figure of a cross-section of the stele from the young stem of *Marattia* which shows that it has essentially the same structure as that of *Danæa*. There is a fairly well-marked endodermis, within which lies a broad zone of phloem, entirely surrounding the central mass of xylem.

According to Kühn, there is in the older plant a second circle of bundles within the first, but no satisfactory account is given as to the relation of the bundles making up this second circle with those of the outer dictyostele. However, it is probably composed also of elements derived from the leaf traces, but it is possible that some of the strands may be of cauline origin.

### THE ADULT SPOROPHYTE OF MARATTIA.

The genus *Marattia* includes about 25 extremely variable species, some of which, e. g., *M. fraxinea*, closely resemble *Angiopteris* in their general habit (see Bitter 1, page 441; Christensen 1). They occur in the moist, tropical regions of both the Old and New World, and one species, *M. salicifolia*, extends as far south as the Cape Region of South Africa. *M. douglasii* is a conspicuous fern of the Hawaiian Islands. This species I have studied somewhat in detail, as well as the West Indian *M. alata*, which is abundant in the mountain forests of Jamaica. These two species closely resemble each other in general habit and have the leaves very much more divided than is the case in *M. fraxinea* and its allies (see plate 12).

The stem in the adult plant is an almost globular, upright caudex, a foot or more in diameter in large plants. The closely set, spirally arranged leaves have very stout petioles, 5 or 6 centimeters in diameter at their base, which is enlarged and provided with two very large wing-like fleshy stipules, which, with the base of the leaf, remain attached and completely cover the caudex after the petioles have fallen away. There is at the base of the leaf, as in the other Marattiaceæ, a pulvinus-like enlargement, where the leaf breaks off, leaving a clean scar marked by the broken ends of the vascular bundles. The leaves may reach a length of 2 to 3 meters or

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even more. The thick, fleshy texture of the leaflets, much like that of *Botrychium ternatum* or *B. silaifolium*, at once distinguishes *Marattia* from any of the large leptosporangiate ferns with which it may be associated. The resemblance to the leaves of *Botrychium* is especially marked in the young plants growing from the stipular buds. These leaves show a marked triangular outline, curiously similar to that of the larger species of *Botrychium* (see plate 12, B). The venation of the leaflets is also very like, resembling that of *Botrychium* more than it does that of *Danæa* or *Angiopteris*. In the species with large linear leaflets, e. g., *M. fraxinea*, the form of the leaf is quite similar to that of *Angiopteris* or *Danæa*, but the veins are not so closely approximated.

The structure of the leaf was particularly studied in *M. alata*, which closely resembles the Hawaiian *M. douglasii*. In well-grown specimens the leaves measure 2 or 3 meters in length, with a petiole which is 5 or 6 centimeters in diameter above the insertion of the stipules and is somewhat larger lower down. Where it joins the stem there is a marked constriction and the leaves often become broken off at this point, the fleshy leaf base remaining alive and often giving rise to adventitious buds (plate 12, A, 3). A section of the leaf base above the stipules shows the vascular
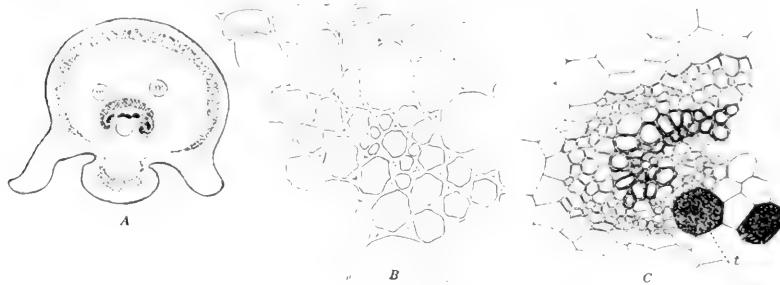


FIG. 176.

A. Section of ultimate rachis of a leaflet of *Marattia alata*. The shaded area is collenchyma; *m*, mucilage ducts.
B. Collenchyma cells, more highly magnified.
C. Part of vascular bundle. The shaded cells are tannin sacs.

bundles to be arranged in two large concentric circles, within which is a smaller third ring corresponding to the two inner bundles found in the section of the younger leaf. Below this point there are numerous anastomoses of the bundles before they enter the cortex of the stem, and the leaf trace consists of a much smaller number of bundles, eight in the specimen examined, which are arranged in a single circle, open on the adaxial side. The distribution of the bundles to the stipules was not studied, but it no doubt corresponds to that in other species, where the veins form an extensively branched system, connected with the bundles of the petiole.

The large leaves of *M. alata*, when fully developed, are five-pinnate, the ultimate leaflets being about 2 centimeters long. From the midrib extend lateral veins which usually fork once but may remain undivided (plate 12, A, 1). The bundles of the rachis in the later divisions of the leaf diminish in number and show the horseshoe-like arrangement seen in the leaf trace after it leaves the petiole. In the final divisions the crescent of bundles seen in the larger rachis is always completely united and in section appears as a single horseshoe-shaped bundle (fig. 176, *A*). In the main stipe and secondary and tertiary rachis there is a conspicuous hypodermal sheath of sclerenchyma. This sclerenchyma passes into collenchyma near the base of the

stipe and finally disappears entirely before the stem is reached. The sclerenchyma is also replaced by collenchyma in the rachis of the terminal divisions of the leaf. Tannin sacs are quite absent except from the immediate vicinity of the bundles, where they occur upon the inner concave side, either in direct contact with the bundle or actually within it. The leaf lamina is only about half as thick as that of the leathery leaf of *Danæa elliptica*, but shows much the same structure. The palisade cells are not quite so well developed and there is a complete absence of sclerenchyma, but otherwise the structure is very similar. The cells of the epidermis are undulate in outline and the stomata, like those in *Danæa*, are surrounded by a series of accessory cells.

The large roots may attain a diameter of 6 to 7 millimeters. In the absence of the hypodermal sheath of sclerenchyma they more nearly resemble *Kaulfussia* than *Danæa*, but they are more like *Danæa* in the greater number of xylem rays, which number twelve in the largest roots examined. In these later roots the central part of the stele is occupied by the pith, the xylem rays not being united as they
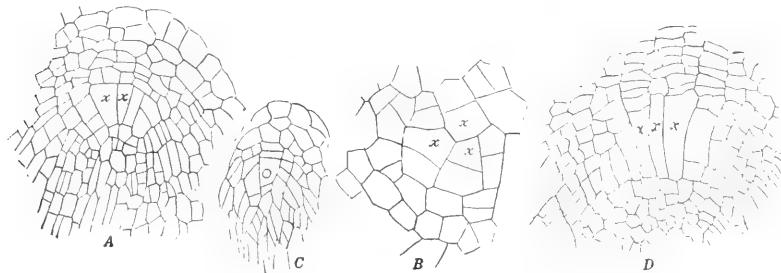


Fig. 1**.

A. Apex of a small root of *Marattia douglasii*, showing two initial cells.
B. Transverse section of apex of a similar root.
C. Longitudinal section of the apex of the second root.
D. Apex of a large root of *M. alata.   x x*, initial cells.   ×200.

are in the roots from the younger plant, but in some cases a few scattered tracheids appear in the central region. There is usually a ring of conspicuous mucilage ducts in the cortex, but these were sometimes not very well developed. Numerous tannin sacs are scattered through the cortex and also occur within the stele.

## THE SPOROPHYTE OF ANGIOPTERIS.

*Angiopteris* is the largest and most specialized of the Marattiaceæ. As we have indicated before, there is much difference of opinion as to the number of species which should be recognized and many botanists consider that all of the forms belong to a single extremely variable species. Bitter, however, in his account of the Marattiaceæ in the "Natürliche Pflanzenfamilien," thinks that from 20 to 30 species should be recognized, following in this respect the classification given by Presl and De Vries; while Christensen (Christensen 1) recognizes 62. I have examined material from Australia furnished by Mr. J. H. Maiden, director of the Botanical Gardens in Sydney, as well as material collected by myself in Ceylon and Java. Some of the specimens growing in the Botanical Garden at Buitenzorg are the largest that I have seen. Leaves 6 meters and upwards in length were measured and the upright caudex was almost as big as a barrel. The specimens seen in Ceylon were somewhat smaller.

Forms of *Angiopteris* occur from Polynesia to Madagascar and northward as far as the Himalayas. The genus also occurs in northern Australia.

The anatomy of the adult plant has been investigated more thoroughly than that of any other member of the Marattiaceæ. The most recent account of the anatomy is that given by Miss Shove (Shove 1), while Farmer and Hill have carefully investigated the vascular system of the young sporophyte. A number of preparations were made by the writer for the purposes of comparison with the other genera, but no attempt was made to follow out in detail the extremely complicated vascular skeleton of the adult sporophyte. Farmer and Hill (Farmer 3) have given a detailed account of the development of the vascular system in the young sporophyte of *Angiopteris*, so that it was not thought necessary to make a large number of preparations of this species, the material of which was collected at the same place where Farmer obtained his plants. However, as these authors did not trace the development of the young bundles from the apical meristem, it seemed worth while to examine this point for the purpose of supplementing their account based upon the study of the fully developed vascular skeleton.
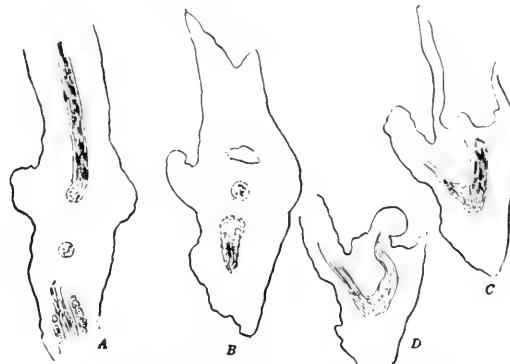


FIG. 178.—Four longitudinal sections of a young sporophyte of *Angiopteris*.

The vascular system begins, as was recognized by Farmer in an earlier paper (Farmer 1), as a single strand connecting the root and cotyledon, exactly as is the case in the other Marattiaceæ. In the further study of the development of the vascular skeleton Farmer and Hill employed the method of constructing a model of the vascular system, built up of superimposed sheets of wax, corresponding with the outline of the vascular bundles seen in the serial sections. The figures which they give, drawn from such models, show very clearly the relation of the vascular strands which make up the complicated skeleton in *Angiopteris*. The assumption is made that the single stele found in the young plant is really a cauline structure, the leaf traces being subsidiary. The early history of the vascular system of the young sporophyte is given by them as follows:

"The vascular skeleton in the young plant of *Angiopteris* consists of an axile rod of tissue, from which strands are given off to the roots and leaves respectively. The first lateral root is given off at a point not quite opposite the formation of the first leaf trace. It is separated from it by about 130°. The regular relation between the leaf and the corresponding root is, however, soon lost. The gaps produced by the early leaf traces are very small and are immediately made good above. The

first deeply depressed aperture or gap occurs at about the sixth or seventh leaf. The leaf traces still continue to issue from the stele as *single* strands till a varying number have been formed, but they begin to bifurcate while still within the cortex of the stem.

"As the stem increases the leaf traces become more numerous and crowded and they take away a larger portion of the vascular tissue from the axile strand. The result is that the leaf gaps become less rapidly repaired. The stele is already hollow in this region, that is, it consists of a cylindrical vascular mass with perforations corresponding to foliar gaps and inclosing a core of parenchyma. Sooner or later, the gap above one leaf fails to be repaired until after the exit of the trace of the next leaf and then the original vascular cylinder becomes broken up and assumes a condition, in transverse section, conforming with that of polystely or dialystely."

There is thus a gradual transition from the solid stele found in the earliest stages to a hollow cylinder or siphonostele with a core of pith, and by the formation of larger and larger leaf gaps there is a transition to an open dictyostele like that found in the other genera. Finally there appears the commissural strand which forms a thick
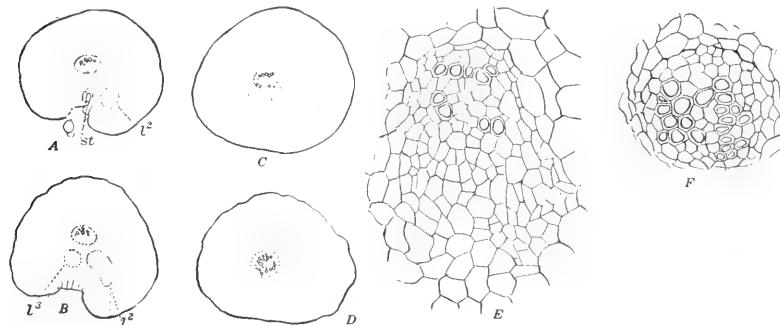


FIG. 179.

A-D. Four transverse sections of a very young sporophyte of *Angiopteris*. A, traverses stem apex. B, C, show the fusion of the first three leaf traces.
E. Central region of C. ×200.
F. Central region of D. ×200.

strand traversing the pith and undoubtedly of cauline origin. This central strand becomes more and more important as the plant develops and from its central position might be mistaken for the original axial strand of the young sporophyte. The vascular system at this stage is described as follows:

"The leaf traces become more complex and anastomoses take place at irregular intervals with the strands which can still be recognized as the relics of the original siphonostele, as well as with one another. Irregularities also commence to become apparent as to the relative heights at which the two members of the leaf traces become freed from the plexus of tissue, and a stage is thus reached at which the vascular skeleton appears to consist of a stout axile strand, surrounded by upwardly diverging zones of steles, which ultimately pass out above to the leaves."

It is evident that at this stage there is a condition which is very much like that figured by Kühn for *Kaulfussia*, and it is pretty clear that the whole of the dictyostele, exclusive of the axial or commissural strand, is made up of the "upwardly diverging zones of steles which pass out above into the leaves," i. e., in other words, the dictyostele is composed of a union of multiple leaf traces.

Transverse sections of a very young sporophyte of *Angiopteris* show the same relation of the parts as in the other genera. The junction of the traces of the first, second, and third leaves takes place at a very short distance below the stem apex, which, as Farmer pointed out, shows an unmistakable apical cell of somewhat irregular form. Compared with the other genera, the primary leaf trace in *Angiopteris* has the xylem better developed, and composed of about half a dozen tracheids, where the trace enters the stem. This primary leaf trace is concentric, as it is in the later leaves, and not collateral, as it is in *Danæa* (fig. 179).

Near the point of the junction of the three primary traces, there may be seen in the young traces from the second and third leaves the first tracheary tissue, consisting of one or two tracheids. As the sections are examined lower down the tracheary tissue of the second and third leaf traces increases in amount until it forms a solid band, separated from the corresponding xylem of the primary leaf trace by a band of parenchyma. The three leaf traces are now completely fused, but the sections of the two xylems are perfectly evident, and this band of tissue between
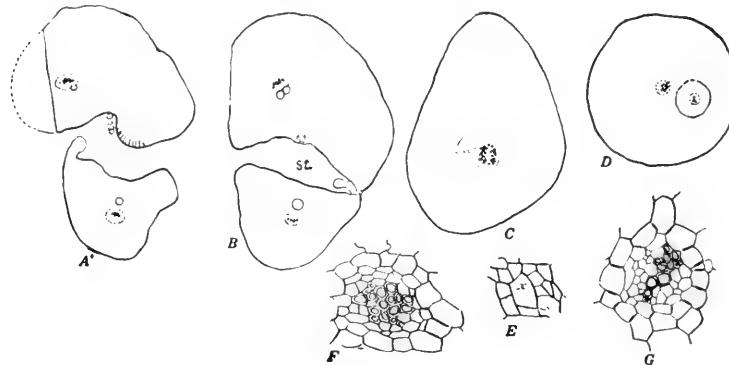


FIG. 180.

A–D. Four sections of an older sporophyte than that shown in fig. 179. ×40.
E. Stem apex. ×130.
F. Central bundle from transitional region.
G. Bundle of primary root.

the two xylems (which really belongs to the ground tissue of the stem and not to the stele itself) corresponds to the "pith" figured by Farmer and Hill for the transitional region between the root and stem (fig. 180, *C*). Sometimes, in still lower sections, the two xylems are connected, but I have found no cases where the pith was entirely surrounded by the xylem, and the two xylems of this axial strand merge gradually into the two xylems of the diarch primary root (fig. 179, *G*). The endodermis is clearly evident in the intermediate region and is recognizable also at higher levels, but is less easy to distinguish.

In a series of sections, from a plant in which the fifth leaf was just recognizable, a section at the level of the stem apex shows the apical meristem to be composed of several large cells, one of which is probably the single apical cell, but this is not easy to determine. The base of the fifth leaf is close to the apical group and the section of its stele is indicated by a group of small cells close to the stem apex. The stele of the third leaf is clearly seen, separated from the fourth leaf by about one-third the circumference of the stem. Below the stem apex these two leaf traces

approach, but are still separated by a considerable mass of tissue, lying directly under the stem apex. The two bundles finally join and between them there seems to be a sort of connective tissue which may perhaps belong to the stem itself, so that this central stele of the stem may possibly have a certain amount of cauline tissue in addition to that derived from the leaf traces; but it is quite as likely that this connective tissue between the bundles is nothing more than a lateral extension of the leaf trace itself, very much as is the case in *Helminthostachys* and *Botrychium*.
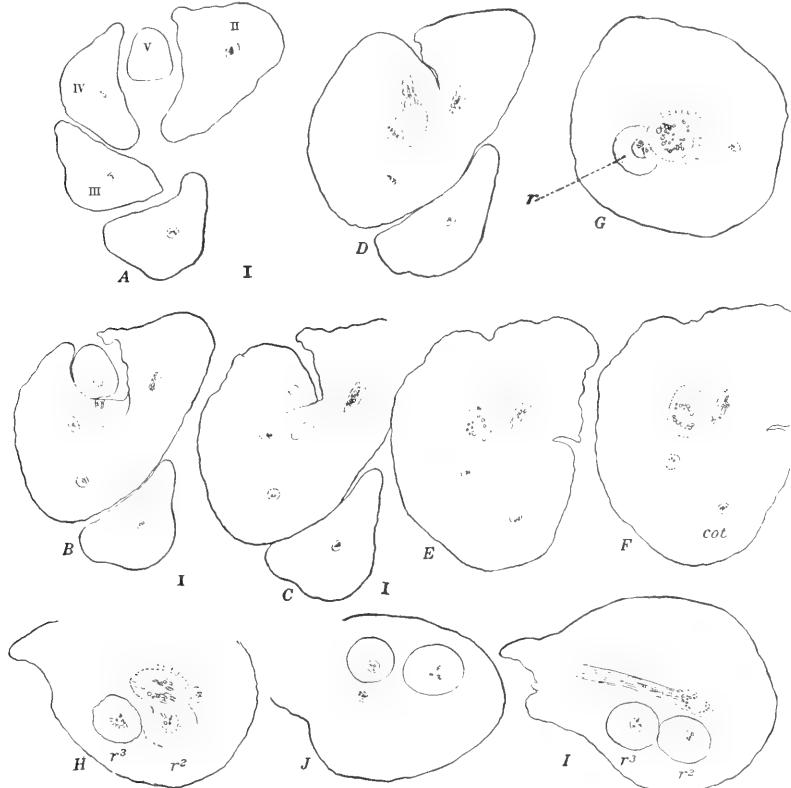


FIG. 181.—Series of transverse sections from a sporophyte of *Angiopteris*, showing 5 leaves. $r^2$, $r^3$, second and third roots. ×25. B, traverses the stem apex.

When the fusion is complete, the section of the stele appears circular in outline and the whole of the tissue is apparently quite uniform, with the exception of a single tracheid marking the position of the xylem of the older leaf trace. There follows almost immediately the older leaf trace which joins the solid stele, formed by the fusion of the traces from the third and fourth leaf, without causing any break. At this level the first tracheary tissue can be seen in the fourth leaf trace also. The structure of the stem below the junction of the second and third leaf traces is the same as that already described for the younger sporophyte. A very young root was

formed just opposite where the second leaf trace joined the stele. The apical cell, which had apparently developed from an endodermal cell, had only undergone the first division. This root was probably not the first lateral root, or if it was, it had formed very much later than is usual. The base of the plant had been somewhat injured and there was what looked like the remains of another root, which was probably the first lateral root.

The oldest specimen of which sections were made had four fully developed leaves and the rudiments of the fifth and sixth. An examination of the lower series of sections showed the remains of one or two other leaves and it was probable that eight leaves all together had been formed. At the level of the stem apex (fig. 181, $B$) six separate leaf traces could be seen and the relation of the younger ones to the apex was exactly the same as in the younger sporophyte. Below the apex, the central stele of the stem shows a broken ring of procambium inclosing larger parenchyma, representing the pith. The procambium ring is made up evidently of two portions, one of which is certainly referable to the sixth leaf trace, while the opposite one perhaps represents the beginning of the next leaf trace, although the leaf to which it belongs is not yet evident above (fig. 181, $D$). The junction of the leaf traces so close to the stem apex makes this point very difficult to decide.

The fusion of the fifth and fourth traces follows quickly and the resulting stele shows plainly the three separate xylems of its component bundles. The older portion of the stele shows that it is still solid, with no evident leaf gaps (fig. 181, $E$-$G$), but with several groups of tracheids probably corresponding to as many leaf traces, though less distinct than in the younger plant. This is complicated by the fusion of the root traces, which disturbs the arrangement of the xylems from the leaf traces, but the xylem is less compact than is figured by Farmer and Hill and the "pith" much less definite; indeed, one can hardly speak of a pith in this connection. No trace could be seen of the central commissural strand which later makes its appearance and which presumably arises in the same way that it does in $Danæa$.

In the early stages $Angiopteris$ appears to agree closely with the other Marattiaceæ in the development of its vascular system, but the single central stele without leaf gaps is retained much longer than in the other genera and it also becomes much larger and has a better-developed xylem, and the open dictyostele, formed from the anastomosing of the early single leaf traces, characteristic of $Danæa$ and $Marattia$, is not present. It is not quite clear whether the "siphonostele" with its small leaf gaps, which is the next stage in the development, is made up entirely of leaf traces, and it is possible that the stelar tissue, connecting the adjacent leaf traces, may be composed in part of cauline tissue. On the other hand, it is quite as likely that the connection of the leaf traces is brought about merely by a broadening at the point of contact, such as occurs in $Helminthostachys$. Indeed, at this stage, the stele of $Angiopteris$ is more like that of $Helminthostachys$ than like that of the other Marattiaceæ.

#### THE ADULT SPOROPHYTE OF ANGIOPTERIS.

In habit $Angiopteris$ closely resembles the larger species of $Marattia$. The enormous leaves are arranged spirally about the thick upright caudex, which is covered completely by the persistent bases of the old leaves. The leaves are usually twice-pinnate; the linear leaflets have more or less conspicuously serrate margins with a venation very much like that of $Danæa$, the veins being more closely set than in $Marattia$. In addition to the ordinary subterranean roots, large aerial roots are not infrequent, but it is doubtful if there is any essential difference between the two kinds of roots.

The anatomy of the stem closely resembles that of *Marattia*, but the bundles seen in section are much more numerous and instead of being arranged in two circles, exclusive of the central commissural strand, are usually arranged in four or five. The first complete study of the arrangement of the bundles in the stem was made by Mettenius (Mettenius 2). A summary of his results is given in the paper by Miss Shove. Briefly stated, Mettenius describes the distribution of the bundles as follows (Shove 1, p. 498):

"The vascular bundles form funnel-shaped zones, with their lower ends in the axis of the stem and their upper portions continued out into the leaves as leaf traces. It is the transverse sections of these concentrically arranged funnels which appear as the rings of separate bundles in the section of the stem. Segments from the outer zone pass into the leaves as the leaf traces and the gaps thus left are filled up by corresponding segments from the next inner zone."

The detailed account made by Miss Shove was based upon a moderate-sized specimen from Ceylon. This stem is described as being somewhat dorsiventral in structure, which is certainly not usual in *Angiopteris* and may perhaps be explained by the conditions under which the plant was growing. I found *Angiopteris* growing frequently upon steep banks and it is quite possible that plants growing in such a position might be obliged to bend upward, in which case a somewhat dorsiventral structure would be developed. Plants growing upon level ground, so far as my observations go, are always strictly radial in structure.

Miss Shove found essentially the same structure as that described by Mettenius, except that she states that the leaf traces are developed exclusively from the strands of the outer zone, while Mettenius states that strands are also contributed to the leaf trace from the second zone. The following is taken from her paper (Shove 1):

"The general scheme of the arrangement of the vascular tissue in *Angiopteris* is almost clearly conceived by considering it in connection with the insertion of the leaves. The leaf bases, which are set in a rough spiral on the stem, show in their lower parts a meshed segment of vascular tissue having the form of part of the surface of a cylinder. This segment passes from the leaf base into the outermost zone of the stem, uniting right and left with the strands of this zone. Then, continuing in an obliquely descending direction, it passes on into the second zone and so on until it reaches the longitudinal axis of the stem, where it unites with other leaf-trace bundles and loses all individuality."

Miss Shove found that the steles in the stem were both mesarch and endarch in structure. The protoxylem is found in groups of two or more spiral tracheids, some of them at the periphery and some in the center of the stele. The number of protoxylems varies with the size of the stele, the larger ones usually containing five or six such groups. The earliest protoxylem appears at the periphery of the stele. Protophloem is developed upon the outer side of the stele in the form of discontinuous, small, thick-walled elements, which Miss Shove regards as sieve tubes. This protophloem does not occur upon the inner side of the xylem. A remarkable peculiarity of the bundle is the fact that the secondary sieve tubes are formed outside the protophloem instead of within it. The phloem is of greater breadth on the outer side of the stele than on the inner. No endodermis could be detected about the steles in the stem. The usual absence of sclerenchyma was noted for the stem. Miss Shove was unable to determine the nature of the apical meristem and the question still remains open, whether the stem grows from a single apical cell or from a group, such as is found in the larger roots.

It appears from the account of both the older and more recent investigators that the complicated system of concentric meshed zones in the adult stem of *Angiop-*

*teris* is really built up of leaf traces, the so-called "compensating segment" being nothing more than the lower part of a leaf trace which higher up emerges as a meshed segment from the outer zone and passes into the base of the leaf.

The structure of the petiole (fig. 182, *A*) is like that of *Marattia*, except that the vascular bundles are more numerous and are arranged in several circles, inclosing one or two small bundles in the center. A cross-section of a leaflet (fig. 182, *B*) shows the vascular bundle which traverses the midrib to be horseshoe-shape in section, having a central mass of tracheids, with reticulate or scalariform markings, and surrounded by the phloem, largely made up of large sieve tubes, but having also protophloem cells and bast. The ground tissue is composed largely of parenchyma, but on both sides below the epidermis is a conspicuous band of collenchyma. In the larger divisions of the leaf the collenchyma, as in *Marattia*, is replaced by sclerenchyma. The structure of the lamina is very much like that of *Danæa*, but the palisade parenchyma is even better developed.

A comparison was made of the leaves of two forms (species?) of *Angiopteris*, one from Australia, the other from Ceylon. These showed several notable differences. The leaflets of the Ceylonese specimens were thinner and sharply serrate,
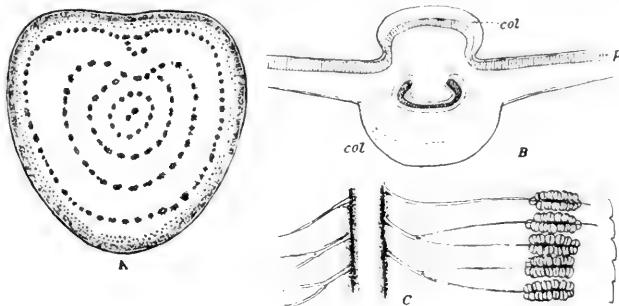


FIG. 182.—*Angiopteris.*

A. Section of petiole from an adult sporophyte, somewhat reduced.
B. Section of a leaflet. *col*, collenchyma; *p*, palisade tissue. ×14.
C. Part of sporophyll, showing the sori. ×4.

while in the Australian specimens the serrations were almost wanting, except at the tapering apex of the leaflet. There were also marked anatomical differences. In the Australian form the palisade cells are very much elongated and are separated from the epidermis by a layer of colorless hypodermal cells, and the spongy mesophyll of the lower part of the leaf is decidedly more compact than in the form from Ceylon. In the latter, the palisade cells are noticeably shorter and abut directly upon the epidermis. Undoubtedly the differences in the anatomy of the two forms are associated with the difference between the moist, tropical climate of Ceylon and the drier and cooler climate of Australia, and perhaps do not necessarily imply that the two species are distinct, although it is highly probable that such is the case.

The roots originate, for the most part, in the inner zones, but a few may arise in connection with the bundles of the outer zones and must necessarily traverse a very large amount of tissue before they finally emerge.

Russow (Russow 1) described two sorts of roots—earth roots which were branched and had but five xylem rays completely lignified to the center of the bundles, and aerial roots which were much larger and unbranched and had twelve to

twenty xylem rays, only the outer tracheids being lignified.  In the plant which was examined by Miss Shove only the earth roots were found, and these, instead of having only five xylems as Russow asserts, had from ten to thirteen and the xylem elements were entirely lignified.  The probabilities are that there is no sharp line to be drawn between the aerial roots and the earth roots.  Figure 183 shows a section of a root from the base of a small plant from Australia, in which there were fourteen xylem masses.  As will be seen, there is the same circle of large mucilage ducts and the tannin cells that are found in the root of *Marattia*, which it very closely resembles.  Root hairs are nearly or quite absent, in which respect these resemble the older roots of other Marattiaceæ.  In two cases Miss Shove found a dichotomous branching of the root.  This, while probably anomalous, is interesting, as it recalls the method of branching in the roots of *Ophioglossum*.

### ARCHANGIOPTERIS.

The genus *Archangiopteris*, with a single species, *A. henryi* Christ and Giesenhagen, is at present known only from a single locality in southern China.  *Archangiopteris* in habit resembles a large *Danæa*.  The leaves, which reach a length of
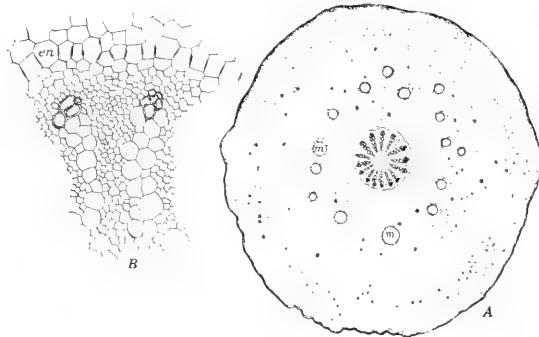


FIG. 183.—*Angiopteris.*
A. Section of a large root.  *m*, mucilage ducts.  ×14.
B. Part of the central cylinder of the root.  *en*, endodermis.  ×70.

about a meter, have from seven to twelve leaflets, much like those of *Danæa elliptica*, both in form and venation.  The stalk of each leaflet is swollen in a manner that suggests the nodular swellings in the petiole of *Danæa elliptica* (fig. 184).

The only account of the structure of the plant is that of Gwynne-Vaughn (Gwynne-Vaughn 2), but as he had only a fragment of a stem he was not able to make a complete study of the vascular system.  To judge from the fragment of the stem which he examined, the leaves seem to be arranged spirally and the stem is probably radial in structure.  The general structure of the leaf base seems to be most like that of *Kaulfussia*.  The leaf trace consists of only two vascular strands, which divide up later into several (eight or nine) separate strands, arranged at the base of the leaf in a horseshoe curve, quite like that of *Kaulfussia*.  The arrangement of the vascular system of the stipules is probably the same as in the other Marattiaceæ.  The structure of the stem is also apparently very much like that of *Kaulfussia*, a section showing only one circle of bundles with a single central strand.  The root, which is like that of the other Marattiaceæ, has from seven to ten xylem rays.

## MACROGLOSSUM.

This represents a new genus of Marattiaceæ, the one species of which, *M. alidæ* Copeland, has recently been described (Copeland **1**). It is a large fern from Borneo, and is evidently related to *Angiopteris*, from which it differs mainly in its simply pinnate leaves, which may reach a length of about 3 meters (see Copeland **1**, plate V).

## TISSUES OF THE MARATTIACEÆ.

There are often present upon the leaf bases of the Marattiaceæ peculiar lenticel-like structures to which German writers have given the name "Staubgrübchen." These arise beneath the stomata, and form small cavities whose peripheral cells become detached and dried up, forming a dust-like powder.

Rod-shaped bodies, mainly composed of cal-cium pectate, are of common occurrence in the intercellular spaces of the tissues of the Marat-tiaceæ. Siliceous deposits and crystals of calcium oxalate have also been observed in *Angiopteris* (see Bitter **1**).

The elements of the vascular bundles are much like those in the bundles of the ordinary ferns. The tracheary tissue is composed mainly of scalariform elements, and the sieve tubes have numerous lateral sieve plates, like those of the other ferns. Hill and Farmer have noted a slight secondary formation of wood in *Angiopteris*, somewhat similar to but much less marked than that in the stem of *Botrych-ium virginianum* (see Hill **1**, Farmer **3**).

## THE SPOROPHYLL OF THE MARATTIACEÆ.

The Marattiaceæ differ most strikingly from the Ophioglossaceæ in the character of the sporo-phylls. In all of the living Marattiaceæ the sporan-gia or synangia are borne upon the lower surface of the leaves, which usually are not at all different



Fig. 184.—*Archangiopteris henryi* (after Christ and Giesenhagen).

from the sterile ones. In *Danæa* (fig. 189) the sporophylls are decidedly contracted and the very large synangia almost completely cover the lower surface of the leaflets, but in the other genera the sporophyll is not at all contracted and much the greater part of the leaf surface is free (figs. 182, 184, 185, 188).

*Kaulfussia* (fig. 188) differs most from the other genera, the very peculiar cir-cular or rarely oval synangia being scattered apparently without any definite order over the whole lower surface of the leaf. In *Danæa* the elongated synangia, which lie over the veins, extending from the midrib nearly to the margin of the leaflet, are crowded so that they leave very little of the surface free. In *Marattia* and *Angiop-teris* the synangia lie over the veins as they do in *Danæa*, but they are very much shorter and are formed near the margin of the leaflet, within which they form a single line which occupies only a very small part of the leaf. *Archangiopteris*, except that the synangia are very much longer, agrees closely with *Angiopteris*.

No proper indusium is present in the Marattiaceæ, although very commonly about the base of the synangium there are found a few hairs or scales which have sometimes been considered to represent an indusium. In *Danæa* there is a growth of tissue between the elongated synangia, which grows up into a sort of ridge, the

top of which is expanded so that it overarches the synangia. This ridge in section appears T-shape. Whether this growth of leaf tissue between the synangia in *Danæa* is to be considered as an indusium may be questioned. It appears to be very similar in structure to the oblong cavity or fovea, within which is sunk the not very dissimilar synangium of *Isoëtes* (fig. 189, *B*).

<p style="text-align:center">THE SPORANGIUM OF THE MARATTIACEÆ</p>

In *Angiopteris* and *Archangiopteris* there are formed separate sporangia not very unlike those found in *Botrychium* or *Helminthostachys*, but in all the other genera the sporangia are fused into a synangium which might be compared with the spike of *Ophioglossum*. The synangium in *Danæa simplicifolia* may reach a length of over 3 centimeters and contain upward of 100 loculi. The first study of the development was made upon *Marattia* by Luerssen (Luerssen **3**). According

to his statement, the differentiation of the synangium begins while the leaf is still very small and rolled up between the stipules. The tissue about a vein begins to develop into an elevated cushion following the vein. Upon this receptacle there are developed two parallel ridges of tissue, separated by a cleft. These two ridges later grow upward and meet above, so that their edges completely close up the cleft, which no longer shows from the outside. In each half of this synangium, very much as is the case in the sporangial spike of *Ophioglossum*, there are developed separate archesporial groups corresponding to the separate chambers that are found in the fully developed synangium. Luerssen states that the whole process takes about six months for its completion (fig. 186).



FIG. 185.—*Marattia douglasii*.
A. Leaf from a young sporophyte. *st*, stipules. ×2.
B. Leaflet with synangia. ×4.
C. Horizontal section of a synangium. ×10.

In *Angiopteris* (fig. 187) the development of the sporangium begins at a much later period, when the leaf is almost completely developed. The sporangia here are arranged in an oblong group or sorus, which corresponds to the synangium in *Marattia*. The formation of the sorus begins, according to Goebel, as an oblong depression above a young vein, and about this depression there is formed a circle of short hairs which are sometimes supposed to represent an indusium. Two ridges corresponding to those found in the young synangium of *Marattia* are formed, but upon these the young sporangia develop separately, very much as they do in *Botrychium*.

Goebel states that the archesporium in *Angiopteris* can be traced back to a single hypodermal cell. This cell divides repeatedly but apparently without any definite order, and there is finally formed a large mass of sporogenous tissue, each cell of which gives rise in the usual way to four spores. The cells about the archesporium develop the tapetum. Goebel states that these tapetal cells are destroyed before the division of the spore mother cells, but I have found that this is not the

case and that the tapetal cells persist until the division of the spores is complete. Bower has confirmed this statement and found that the same condition of things obtains in the other genera.

The most complete account of the development of the sporangium is that of Bower (Bower **6**), who has studied the development in all of the genera except *Archangiopteris*. Except for the difference in form, there is no essential difference in the development of the sporangia in *Kaulfussia* and *Danæa* from that found in *Marattia*. In *Danæa*, however, the individuality of the loculi of the synangium is less clear than in the other genera. Very often Bower found that the arche-sporium became divided more or less completely by parts of the sterile tissue, some-what in the same fashion that the so-called "trabeculæ" are formed in *Isoëtes*. In *Danæa*, moreover, the synangium from the first is solid, and the cleft which is present in the young synangium of *Marattia* is absent. In *Kaulfussia* the develop-ment of the synangium differs in that a single circular ridge is formed instead of the
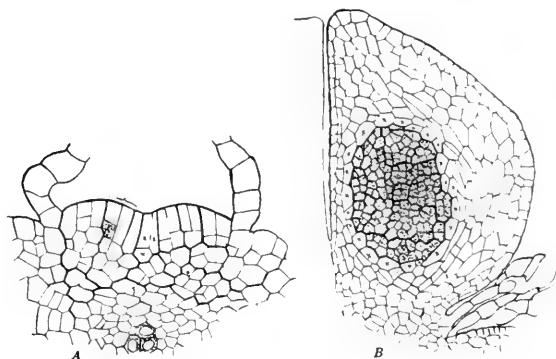


FIG. 186.
A. Section of a young synangium of *Marattia fraxinea*. ×225.
B. Loculus from an older synangium. x, the tapetum (after Bower). ×112

two parallel ones found in *Marattia*, and the loculi or chambers of the synangium are thus arranged in a circle around a central pit-like depression. Bower states that the sporogenous tissue of each loculus in all the forms he examined can usually be traced to a single mother cell. He also found that the tapetum always arises from the cells adjacent to the archesporium, and that normally all of the sporogenous tissue develops into spores. In these respects the Marattiaceæ closely resemble *Helminthostachys* and *Botrychium*.

In *Danæa* and *Kaulfussia* there is no mechanical tissue representing the annulus found in the more specialized ferns. The dehiscence of the sporangium in these forms is brought about merely by a shrinking of the cells on either side of the slit by means of which each loculus opens. This slit is very short in *Danæa* and may finally appear as a circular pore, but it is not essentially different from the more elongated slit found in *Kaulfussia* and *Marattia*. In the latter there is developed, in the outer tissues of the synangium, mechanical tissue which causes the two halves of the synangium to separate, very much like the two covers of a book, and the elongated slit from each loculus opens into this space between the widely separated halves of the synangium.

The dehiscence of the individual loculi is affected by the contraction of the thinner-walled cells surrounded by firmer tissue. The sporangium of *Angiopteris* is undoubtedly a more specialized structure than the synangium of the other Maratti-aceæ. In *Angiopteris* each individual sporangium has the wall on the outer side much thicker than that on the inner one, and the superficial cells have their walls much thickened. The inner wall is sometimes composed of but one layer of cells outside of the tapetum, but more commonly there are one or two layers of cells between the tapetum and the epidermis. Near the top of the sporangium on its outer side there is a transverse band of cells with thicker walls, and these constitute a rudimentary annulus very much like that found in the Osmundaceæ. By the contraction of this thickened annulus the longitudinal slit on the inner face of the sporangium is made to open widely at maturity. The number of spores produced in each loculus, according to Bower, is approximately 1,750 for *Danæa*, 7,500 for *Kaulfussia*, 2,500 for *Marattia*, and 1,450 for *Angiopteris*.

Which type of sporangium in the Marattiaceæ is the more primitive is very difficult to say, as both the free sporangium like that of *Angiopteris* and the compact



FIG. 187.—*Angiopteris*.

A, B, sections of young sporangia (after Goebel). C, an older sporangium. *r*, the annulus; *t*, the persistent tapetum. ×75.

synangium like that of *Marattia* and *Danæa* are of about equal antiquity, so far as the geological record goes. It must be remembered that the living Marattiaceæ are almost certainly merely a few isolated fragments of a once large group, and it is by no means necessary to assume that the sporangia of the living forms must necessarily all conform to a common primitive type. It seems quite as likely that the free sporangia, like those of *Angiopteris*, may have originated directly from some ancient prototype which resembled, perhaps, forms like *Botrychium* or *Helminthostachys*, while the genera with the solid synangium like *Danæa* may have come from forms with completely united sporangia like *Ophioglossum*.

It appears from a study of the most ancient ferns that these had dimorphic leaves, the sporophyll probably resembling the fertile spikes of *Botrychium* or the fertile leaf segments of *Osmunda*. How the modern Marattiaceæ originated from forms of this type is not by any means clear. The development in *Helminthostachys* of sterile leaf-like lobes associated with the sporangia may perhaps afford a clew to the method of sterilization by which the sporangiophores of some type allied to the Ophioglossaceæ may gradually have developed sterile green leaf segments, bearing upon their lower surface sporangia or synangia like those of the modern Marattiaceæ. These sterile segments found in *Helminthostachys* are occasionally strikingly leaf-like, and as the groups of sporangia in *Helminthostachys* are sometimes united into small synangia, the development of similar synangia upon the lower surface of such green sterile segments of the sporangiophore is quite conceivable.

Another explanation might be the coalescence of the fertile and the sterile parts of a sporophyll like that found in the Ophioglossaceæ, but the fact that the spo-

rangiophore in all of the living Ophioglossaceæ is adaxial, while in the Marattiaceæ the synangia are abaxial, is a serious objection to such a hypothesis; and the most plausible explanation at present seems to be that of a progressive sterilization of part of the sporophyll itself and the expansion of the sterile tissue into the broad lamina of the leaf bearing the separated sporangia or synangia upon its lower surface.

It must be admitted that the difference between the sporophylls of the existing Marattiaceæ and those of the Ophioglossaceæ is very great, and the leaves of the former, superficially at least, are much more like those of the leptosporangiate ferns; but the sporangia themselves are very similar in their development to those of the
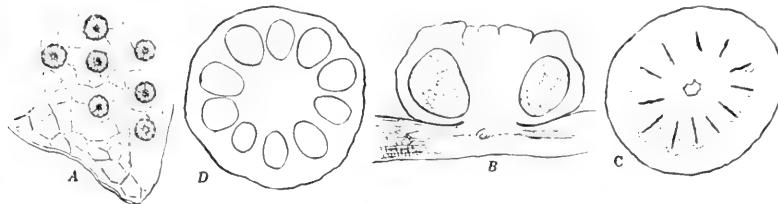


FIG. 188.

A. Lower surface of a sporophyll of *Kaulfussia*, showing          C, D. Cross-sections of a synangium.
      circular synangia.   ×1.5.                                                        C. Near top, showing openings of loculi.
B. Median section of a synangium.   ×12.                                       D. Near middle.   ×12.

Ophioglossaceæ, and offer no obstacle to the assumption of a fairly close relationship between the Ophioglossaceæ and the Marattiaceæ, which is indicated by the history of the development of the gametophyte, and the vegetative organs and tissues of the sporophyte. Perhaps the most marked difference between the tissues of the Marattiaceæ and the Ophioglossaceæ is the prevalence of the mucilage ducts in the tissues of Marattiaceæ, but these arise rather late in the history of the sporophyte, and it is
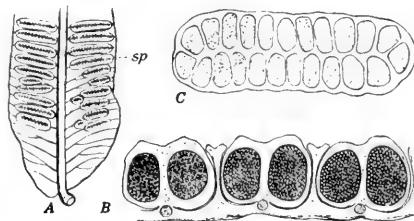


FIG. 189.—*Danæa jamaicensis.*
A. Base of a fertile leaflet, showing synangia, *sp*.   ×2.
B. Transverse sections of three synangia.   ×12.
C. Horizontal section of a synangium.

significant that they are less developed in the presumably more primitive *Kaulfussia* than they are in the more specialized types like *Angiopteris*. Another difference is the development of sclerenchyma, which is quite absent from the Ophioglossaceæ; but this again is also quite absent from *Kaulfussia*, which, on the whole, must be considered to be the most primitive of the living Marattiaceæ and also the form which most closely approaches the Ophioglossaceæ.

# Part III. THE ORIGIN AND RELATIONSHIPS OF THE EUSPORANGIATÆ.

The eusporangiate ferns as they now exist represent but a few isolated fragments of what in earlier geologic time was presumably a very much larger and more connected group. As the geological history of these forms is very far from clear (this being especially true of the Ophioglossaceæ), we are, perforce, dependent mainly upon a comparative study of the few existing types for information concerning their relationships. In the foregoing pages an endeavor has been made to trace the development of these forms, both in their gametophytic and early sporophytic stages, and the result of these studies has been to confirm the belief that a real genetic relationship exists between the Marattiaceæ and the Ophioglossaceæ.

While the three genera of the Ophioglossaceæ differ in certain particulars from each other, there is no question as to their being comparatively closely related, and the same is true of the different genera of the Marattiaceæ, although perhaps the differences here are somewhat greater than in the Ophioglossaceæ.

It has been assumed in these studies that the sporophyte of the ferns is the result of a progressive specialization of the sporogonium of some form allied to the Bryophytes, though it is highly improbable that any of the existing Bryophytes are directly related to this progenitor of the primitive ferns. No attempt will be made here to discuss the reasons for accepting the "antithetic" theory of the alternation of generations, rather than the homologous theory. These reasons have been set forth at length elsewhere.

It is hopeless to expect that any satisfactory fossil traces will be found of these predecessors of the true ferns. The fern type is exceedingly ancient, but it must have been preceded by simpler forms connecting it with some bryophytic type. These forms, as well as the earlier true Pteridophytes, were almost certainly plants of small size and delicate texture, probably not very unlike some of the small and delicate species of *Ophioglossum*. Such plants, composed entirely of soft, perishable tissues, could hardly be expected to leave recognizable fossil traces, and their absence from the ancient Paleozoic rocks is not to be wondered at.

There still exist, however, among the Bryophytes, certain forms which, if they are not directly related to the eusporangiate ferns, nevertheless show many striking similarities in structure, which help to explain at least what may very well have been the character of the liverwort-like ancestors from which the ferns are descended. Among the living Bryophytes, as is well known, an interesting series of types may be traced, showing the gradual increase in the importance of the neutral generation—the sporophyte—starting as little more than a mass of spores, finally by a progressive sterilization of what was originally sporogenous tissue, and an accompanying specialization of the sterile tissues thus formed, attaining a condition of almost complete independence. The importance of this process of sterilization of potentially sporogenous tissue, has been especially clearly expounded in the works of Professor Bower.

In two classes, the true mosses or Musci and the horned liverworts or Anthocerotes, the sporophyte continues its growth for several months and develops an elaborate system of green, assimilative tissue, quite comparable to that found in the vascular plants. The spore-producing function is correspondingly subordinated and the spore formation is delayed until a late period in the life of the sporophyte.

The large, green sporophyte, however (fig. 190, *A*, *sp*), never attains complete inde-
pendence, as no proper root is developed and it is dependent for its water supply
upon the gametophyte.

In all of the Eusporangiates, the embryo is much later in developing its organs
than in the Leptosporangiates, and thus resembles the Bryophytes; moreover, the
embryo reaches a very much larger size before it attains its independence. Even
after the young sporophyte has developed several roots and leaves, it may still



Fig. 190.

A. Gametophyte of *Anthoceros* sp. with the large
sporophyte, *sp*, attached to it. Upper part
of sporophyte is split into valves which allow
the escape of spores.

B, C. Two gametophytes of *Marattia douglasii*, with
attached sporophyte. Sporophyte emerges
upon upper side of gametophyte, very much
as in *Anthoceros*, but a root, *r*, is developed
which penetrates lower side of gametophyte
and connects the young sporophyte with the
earth. *g*, gametophyte; *sp*, sporophyte;
*l*, first leaf; *r*, primary root.

maintain its connection with the gametophyte and be to a certain extent dependent
upon it in a way which forcibly recalls the relation of gametophyte and sporophyte
in the mosses. This condition, which furnishes a very strong argument in favor of
the primitive nature of the Eusporangiates when compared with the Leptosporan-
giates, is especially pronounced among the Ophioglossaceæ, where it may even go so
far that spores are developed before the connection of gametophyte and sporophyte
is finally sundered.

In the true mosses the sporophyte is exceedingly specialized and shows but a
very remote resemblance to that of the Pteridophytes. In *Anthoceros*, however, the
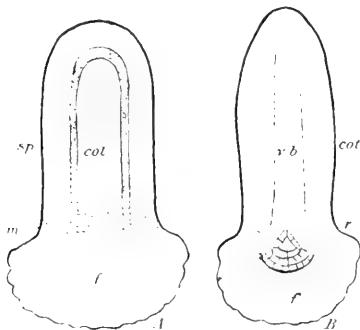


Fig. 191.

Diagrams which show the similarity in form of the very
young sporophyte in *Anthoceros*, A, and *Ophioglossum*,
B. In the former the upper portion becomes an elong-
ated capsule within which spores are produced. The
shaded portion shows layer of sporogenous tissue sur-
rounding central sterile tissue or columella. The
zone, *m*, between capsule and foot, *f*, is composed of
meristematic tissue. In *Ophioglossum* the upper part
of the young sporophyte forms the primary leaf, or
cotyledon, *cot*, which is not sporogenous. The colum-
ella of *Anthoceros* is replaced by the axial vascular
bundle, *v.b.* At the junction of the cotyledon and
foot arises the primary root, *r*, which finally penetrates
the foot and enters the ground.

most highly developed of the horned liverworts, there are a number of very significant
structural details that are very strongly reminiscent of the young sporophyte of
*Ophioglossum moluccanum*. In both of these forms the young embryo has a very
large foot and a conical upper region (fig. 191). This upper portion in *Ophioglossum*
develops into the cotyledon; in *Anthoceros*, into the spore-bearing part of the sporo-
phyte. Above the foot in *Anthoceros* there is developed a zone of meristematic
tissue by means of which new cells are added to the base of the growing sporo-

phyte, and in *Ophioglossum* much the same thing occurs in the cotyledon, although at first this grows from a single apical cell. It is probable that in the ancestors of the Ophioglossaceæ this upper conical portion of the embryo was developed directly into a spore-bearing organ. There is, however, a marked difference which soon appears, viz, the development of a true root in *Ophioglossum*. This root, pushing down through the foot, penetrates the lower side of the gametophyte and places the young sporophyte directly into communication with the water supply from the earth, and thus the latter becomes entirely independent. Were the large sporophyte of *Anthoceros* to develop a similar root from the basal meristem, it also would become entirely self-supporting. Indeed, so marked are the resemblances in the early stage of development that they make the inference almost irresistible that the Ophioglossaceæ must have descended from some simpler forms whose sporophyte bore a strong resemblance to *Anthoceros*. This "pro-*Ophioglossum*," if we may call it so, presumably produced spores upon the first leaf, instead of its being a sterile cotyledon as in the existing forms. From a study of the development of the sporophyte
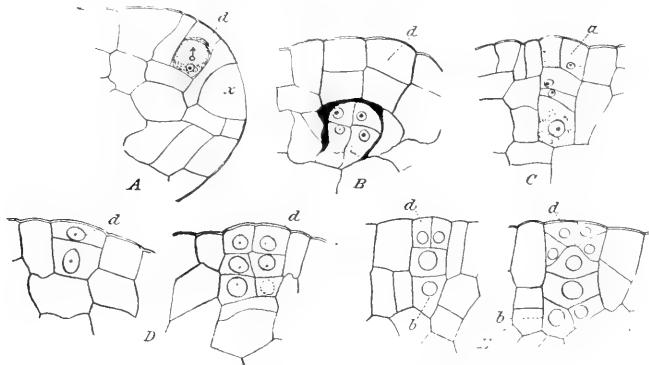


FIG. 192.—Comparison of sexual organs in Anthocerotaceæ and *Marattia*.

A. Longitudinal section of the thallus of *Anthoceros pearsoni* Howe, showing an antheridium mother cell, with the superimposed cover cell (*d*).
B. An older antheridium within a cavity covered by a double layer of cover cells.
C. Young archegonium of *Megaceros tjibodensis* Campbell, a form closely related to *Anthoceros*.
D. Sections of young antheridia of *Marattia douglasii* Baker. The inner cell divides at once into the sperm cells, and the cover remains single-layered. *d*, the cover.
E. Young archegonia of *Marattia douglasii*. *b*, the basal cells of the archegonium; *d*, neck.

in *O. moluccanum*, it may be inferred that this ancestral form had no stem, but consisted simply of this spore-bearing leaf and a root. The sporangia were presumably simpler than in the existing forms of *Ophioglossum*, and perhaps intermediate in character between such sporangia as those of the smaller species of *Ophioglossum* and the imperfectly segregated spore groups which occur in the Anthocerotes.

Not only does the sporophyte of *Anthoceros* show important resemblances to that of *Ophioglossum*, but the development of the sexual organs also shows striking analogies. The archegonium, unlike that of other Bryophytes, is sunk below the surface in much the same fashion as that of the eusporangiate ferns, and the short neck of the archegonium in the latter is probably to be compared to the four terminal neck cells which may occur in the Anthocerotes (fig. 192). The endogenous antheridia of the Anthocerotes also may perhaps be compared to the sunken antheridia of the eusporangiate ferns, and in their early stages the resemblances are very close.

The most marked difference in the character of the reproductive organs is the spermatozoid. The spermatozoids in the Eusporangiates, especially in *Ophioglossum*, are very large and possess numerous cilia, while in *Anthoceros* they are minute and have but two cilia, like the other Bryophytes. This is perhaps the strongest reason for assuming that there is not a direct connection between the Anthocerotes and the Ophioglossaceæ; but both gametophyte and sporophyte have so many points in common that it may be pretty safely assumed that the progenitors of the Ophioglossaceæ were not very different, in appearance at least, from the living *Anthoceros*. Whether the differences in the spermatozoid are secondary remains to be seen, but in view of the extraordinary constancy of the form of the spermatozoids in all of the main groups of the Archegoniates, one would certainly expect that large multiciliate spermatozoids would be found in the ancestors of the Eusporangiates, supposing these should ever be discovered.

There is no question that the subterranean prothallium of the Ophioglossaceæ is a secondary condition, derived from some green gametophyte, probably very much like that of the Marattiaceæ. Not only has the chlorophyll been lost, but in *Ophioglossum* and *Helminthostachys* the dorsiventral form of the gametophyte has been replaced by a radially symmetrical thallus. While this may be partially explained as the result of the absence of light, it must be remembered that the equally subterranean prothallium of *Botrychium* is dorsiventral, although the reproductive organs are borne upon the dorsal surface and not upon the ventral one, as they are in most ferns. Whether this position of the reproductive organs is the result of the conditions of growth or whether it indicates that the green gametophyte from which this saprophytic underground form is descended also bore the reproductive organs dorsally, as most liverworts do, can only be conjectured. In the Marattiaceæ antheridia are not uncommon upon the dorsal surface, and according to Jonkmann archegonia also may occur dorsally. Whether this is normal or is the result of unusual light conditions was apparently not investigated. The formation of archegonia in the ordinary ferns may be induced upon the dorsal side of the prothallium, provided it is illuminated from below. If the illumination is equal on both sides, archegonia will develop both on the ventral and dorsal surface.

Of the investigated species of *Ophioglossum*, *O. moluccanum* and the nearly related and perhaps identical *O. pendunculosum* probably represent the most primitive type. In these species there may be a feeble development of chlorophyll in the gametophyte under certain conditions and the saprophytic habit is much less pronounced than it is in the other species of *Ophioglossum* and *Botrychium*.

The development of an endotropic mycorrhiza in the prothallium of the Marattiaceæ is an interesting suggestion as to the probable beginning of the saprophytic habit which characterizes the gametophyte of the Ophioglossaceæ. There seems to be good reason to suppose that the peculiar type of symbiosis, which is developed so highly in the Ophioglossaceæ, began by the development of a fungus associate in some green gametophyte like that of the Marattiaceæ.

The reproductive organs of the Ophioglossaceæ and Marattiaceæ are very similar indeed. It is probable that the short-necked archegonium found in the Marattiaceæ and in *Ophioglossum* is more primitive than the long-necked archegonium of *Botrychium* or *Helminthostachys*. This conclusion is based upon the assumption that the four-necked archegonium of the ferns is a development of the four terminal cells in the neck of the Bryophyte archegonium, *Anthoceros* most nearly approaching this hypothetical ancestral type. This being the case, the forms with the shortest neck would most nearly resemble this assumed ancestral type.

The question of the canal cells is a puzzling one. In the Marattiaceæ, with the exception of *Danæa*, two neck canal cells, or at any rate two nuclei, are present and the ventral canal cell is conspicuous. In *Danæa*, however, the canal cells are very much less perfectly developed, the ventral canal cells especially being exceedingly difficult to demonstrate. In the latter respect *Danæa* shows a remarkable resemblance to the Ophioglossaceæ, especially to *Ophioglossum*, where the ventral canal cell is equally difficult to demonstrate. This apparent degeneration of the ventral canal cell is probably secondary, as all of the Bryophytes and the other Pteridophytes have the ventral canal cell conspicuous.

The antheridium in all of the Marattiaceæ is almost exactly like that of *Ophioglossum*, while *Botrychium* and *Helminthostachys* differ in having the outer wall of the antheridium more or less completely two-layered. If the antheridium is derived from a type like that of *Anthoceros* (fig. 192), where the antheridial cavity is covered by a double layer of cells, it would seem that *Botrychium* and *Helminthostachys* are more primitive than *Ophioglossum* or the Marattiaceæ in this respect.

Of the Eusporangiates, there is no question that the type of embryo found in *Ophioglossum moluccanum* comes the nearest to the assumed ancestral form. This hypothetical pro-fern may be assumed to have developed simply a spore-bearing organ or sporangiophore, perhaps more or less leaf-like, and a root developed from the inner part of the massive foot in the same way that the root actually does develop in the embryo of the Marattiaceæ and *Ophioglossum moluccanum*. Except that the cotyledon of *O. moluccanum* is sterile, the young sporophyte in this species is actually like this hypothetical type, viz, it is composed simply of root and leaf. It is probable that the fertile spike of *Ophioglossum* is not unlike the primitive sporangiophore, and the ancestral form presumably developed such a spike-like sporangiophore from the first, the sterile lamina being a secondary structure arising perhaps from a basal meristem, like that found in the sporophyte of *Anthoceros*. The early development of the spores in the Ophioglossaceæ is a further indication of their primitive nature. The monophyllous condition which prevails among the Ophioglossaceæ must also be regarded not as a secondary condition but as a primitive one, and a study of the development of the sporophyte lends no support to the theory that we have to do with a reduced strobilus. Of the Marattiaceæ, *Kaulfussia* normally approaches the monophyllous condition more closely than any other genus. The older plants, as a rule, have only two or three leaves expanded at one time and there may frequently be but one. In strong contrast with this is the dense crown of leaves found in *Marattia* and *Angiopteris*, although even in these forms the number of leaves is less than it is in the majority of leptosporangiate ferns. The tendency to the monophyllous condition is shown in the younger plants of *Marattia*, developed from the buds on the old leaf bases, where, as a rule, only one leaf is expanded at a time.

The young sporophyte of *O. moluccanum has no stem*. Without exception, for a considerable time at least, the vascular system of the sporophyte is composed exclusively of tissue belonging to the leaves and roots, the stem apex playing no part in the building up of the vascular skeleton. The theory that there is a special stele belonging to the stem, of which the leaf traces and the root traces are subsidiary structures, is not borne out by a detailed study of the evolution of the vascular system in either the Marattiaceæ or Ophioglossaceæ. In all of these that I have examined the vascular system begins as a single strand common to the primary leaf (or cotyledon) and the primary root, and the more complicated vascular system of the older stem for a very considerable period is built up exclusively by additions of new leaf traces or root steles. This condition is permanent in the Ophioglos-

saceæ. In the Marattiaceæ small cauline bundles (commissural bundles) may be developed at later stages, but these are insignificant compared with the complex of united leaf traces.

The antithetic theory of alternation implies that the sporophylls are older structures than the sterile leaves, and those ferns in which the sporogenous function of the sporophylls is most pronounced may be assumed, other things being equal, to be the most primitive. In this respect, as well as in the usually monophyllous condition, the Ophioglossaceæ are distinctly more primitive than the Marattiaceæ. The sporangiophore or fertile leaf segment is a practically independent structure, being differentiated at an extremely early period in the development of the leaf. In the Marattiaceæ the sporangia are subordinate and the leaves upon which they are borne usually differ in no respect from the sterile leaves. The most marked exception to this is *Danæa*, in which the sporophylls differ decidedly from the sterile leaves and the synangia are much larger than those of the other genera, although the leaves themselves are usually smaller.

Bower thinks that the circular synangium of *Kaulfussia* is the most primitive type among the existing Marattiaceæ, but there is some reason to doubt the soundness of this conclusion; at any rate, on theoretical grounds, the large synangia and contracted sporophylls of *Danæa* must be assumed to be more primitive than the type of leaf found in *Kaulfussia*. In regard to the character of the sporophylls, therefore, none of the living Marattiaceæ can be regarded as being very primitive. All of the oldest fern fossils show the sporangia to be borne upon special leaves or leaf segments, in which the lamina is nearly or quite absent (Scott 1, Bower 9). In this respect, therefore, these ancient fossil ferns were more like the Ophioglossaceæ than like the Marattiaceæ, although their sporangia came nearer to the type of Marattiaceæ. It is by no means impossible that these oldest ferns, e. g., the Botryopterideæ, were related to the Ophioglossaceæ.

The single apical cell found in the stem apex throughout the life of the sporophyte in *Ophioglossum* is probably a more primitive condition than the group of cells found in *Angiopteris*. It still remains to be seen whether the single apical cell found in the other Marattiaceæ in their early stages is persistent throughout life or is replaced, as in the roots, by a group of initial cells.

The single axial strand of collateral structure throughout cotyledon and root may be taken as the starting point for two types of vascular skeleton which have arisen from it. The first is that of *Ophioglossum*, where, with the development of the new leaves, there is built up the wide-meshed cylindrical network or dictyostele, composed of single collateral leaf traces. A further development of the same type results in the much more complicated dictyosteles of the Marattiaceæ, where, moreover, the collateral bundles are replaced by concentric ones. This greater complexity is due primarily to the much larger leaves of the Marattiaceæ, in which the leaf traces are compound. In the earlier stages of these forms, however, we have seen that the young leaf traces are single and the structure of the vascular skeleton exactly as it is in *Ophioglossum*. Of the Marattiaceæ, *Kaulfussia* more nearly retains this primitive condition than the other genera and next to this comes *Danæa*, while further removed from the primitive type is the exceedingly complex skeleton found in the massive stem of *Angiopteris*. The concentric bundles, characteristic of the Marattiaceæ, are presumably of secondary origin and we find that in the early bundles, especially of *Danæa*, a true collateral structure is present.

The second type of skeleton is that found in *Botrychium* and *Helminthostachys*. This is a solid, hollow cylinder with inconspicuous leaf gaps, resulting from the union of the broad leaf traces, which fuse completely to form this hollow stele. That

the cylindrical bundle, or siphonostele, is not due to the formation of a pith within a protostele is clearly shown by the study of the development of the bundle in the young sporophyte of *Botrychium*, where it can easily be seen that the component bundles are separate at first, and that the pith, so-called, of the siphonostele is merely a portion of the ground tissue that is included between them, and which later becomes entirely separated from the cortical tissue. A similar condition of things may be found in tracing the development of the vascular cylinder in the young stem of *Helminthostachys*. The development of the young bundles in *Angiopteris* is more like *Botrychium* than *Ophioglossum*, and this, in connection with the separate sporangia that occur in *Angiopteris*, suggests that perhaps the type of the Marattiaceæ represented by *Angiopteris* may have originated independently from forms like *Helminthostachys*, instead of having had the same origin as those forms in which true synangia are developed.

The essential similarity of the leaf structures throughout the Ophioglossaceæ is sufficiently clear, and the steps in the increasing complexity of the sporophyll can be easily traced in existing forms, ranging from the undivided fertile and sterile segments of *Ophioglossum* to the decompound leaves and much-branched panicle of *Botrychium*. *Helminthostachys* is much the most aberrant of the Ophioglossaceæ and in many respects shows a marked resemblance to the Marattiaceæ. The form and venation of the leaves strongly recall *Danæa* or *Angiopteris*, except that the leaves are not pinnately divided, in which respect they closely resemble *Botrychium*. It must be remembered, however, that the ternate form of leaf characteristic of *Botrychium* and *Helminthostachys* is very generally met with in the early stages of the sporophyte in the Marattiaceæ. The anatomy of the leaf in *Helminthostachys* very closely resembles that of the Marattiaceæ, there being a well-developed palisade layer in the mesophyll, a character which is either wanting entirely or very imperfectly developed in *Botrychium*.

The leaves of the Marattiaceæ, except in *Kaulfussia*, in their form and in the circinate coiling of the young leaves, suggest a relationship with the leptosporangiate ferns rather than with the Ophioglossaceæ. However, the larger species of *Botrychium* and *Helminthostachys* show an approach to this circinate form of the young leaf, this being especially conspicuous in the young sporophylls of *Botrychium virginianum*. With the exception of *Kaulfussia*, the general form and venation are alike in all the Marattiaceæ and have their nearest analogy in *Helminthostachys*. There seems no reason to assume that the stipular sheath in the Marattiaceæ is different in its nature from that of the Ophioglossaceæ, especially *Helminthostachys* and *Botrychium*. The ternate form of the leaf may be persistent in *Kaulfussia*, where, however, the venation is more like that of *Ophioglossum*. Whether this occurrence of both reticulate and pinnate venation in the two families is a case of parallel development, or whether it indicates the connection of the families at different points, it is at present impossible to say.

In the structure of the roots the Marattiaceæ find their nearest ally in *Helminthostachys*, where the number of the xylem rays, four to seven, equals that of *Kaulfussia*, which has the simplest structure among the Marattiaceæ, and, as we have seen, probably on the whole is most nearly related to them. *Helminthostachys* also resembles the Marattiaceæ in the character of the apical growth of the root, where, although there is a single apical cell, such as occurs in the young roots of the Marattiaceæ, its form is more like that of the Marattiaceæ than it is like that of *Botrychium*. The endophyte which occurs in the older roots of the Ophioglossaceæ, as well as in the primary one, I have not found in the older roots of the Marattiaceæ, at least not as a regular thing, although it seems to be always present in the primary root.

The Ophioglossaceæ and Marattiaceæ are alike in the absence of any mechanical tissues in the cortex of the stem. Sclerenchyma, which is well developed in the leaves of most of the Marattiaceæ, seems to be entirely wanting in all of the Ophioglossaceæ, but the collenchyma which replaces sclerenchyma in certain organs of the Marattiaceæ, and is the only form of mechanical tissue in *Kaulfussia*, is occasionally found in the Ophioglossaceæ. A notable case of this is the broad and very conspicuous zone of collenchyma surrounding the root bundle in *Ophioglossum palmatum*. The mucilage ducts so characteristic of Marattiaceæ are apparently quite wanting in all of the Ophioglossaceæ, and the tannin sacs which are a nearly constant feature in the Marattiaceæ are absent in *Botrychium* and *Ophioglossum*, but occur in *Helminthostachys*.

In the general structure of the vascular bundles there are important resemblances. In both families the first-formed tracheids in the young sporophyte are all of the short, reticulate form, and this is retained permanently in *Ophioglossum*, but is replaced later by pitted tracheids in *Helminthostachys* and *Botrychium*. *Helminthostachys* alone among the Ophioglossaceæ shows spiral protoxylem elements like those found in the later bundles of the Marattiaceæ.

It is probable that the collateral bundles of *Ophioglossum* represent the primitive type from which the concentric type found in the Marattiaceæ has been derived. In *Botrychium* and *Helminthostachys*, while the collateral type of bundle is found in the stem, in the petioles the bundles are concentric, as they are in the Marattiaceæ. The collateral type of bundle reappears again in the early stages of the sporophyte in *Danæa*.

*Angiopteris*, which is probably the most specialized of the living Marattiaceæ, has concentric bundles only.

# CONCLUSION.

While the more specialized Marattiaceæ, like *Marattia* and *Angiopteris*, apparently differ very much from the Ophioglossaceæ, it appears from a study of the development of these forms, as well as that of the simpler and presumably more primitive genera *Danæa* and *Kaulfussia*, that the most conspicuous differences are of secondary rather than of primary importance, and the conclusion is justified that the two families of the Eusporangiatæ really arose from the same primitive stock. Of the Ophioglossaceæ, *Helminthostachys* is undoubtedly the form which, on the whole, comes nearest to the Marattiaceæ, although *Ophioglossum* is more like them in the character of the reproductive organs and in the vascular skeleton of the sporophyte. *Ophioglossum* also recalls *Kaulfussia* in the form and venation of its leaves.

While the green gametophyte in the Marattiaceæ is undoubtedly a more primitive structure than the saprophytic prothallium of the Ophioglossaceæ, the sporophyte of the latter is certainly a more primitive one than that of the Marattiaceæ. The monophyllous condition, the early development of the sporangia, and the fact that the whole of the sporangiophore is spore-bearing, all point to this.

The way in which the sporophyll of the Marattiaceæ has been derived from a type like that of the Ophioglossaceæ is not clear, and it is by no means certain that all of the Marattiaceæ have been developed in the same way. The occurrence of fossil forms with sporangia of the marattiaceous type grouped together—much like the sporangia of *Botrychium* or *Osmunda*—suggests that such a condition might have been preliminary to the separation of the sporangia by the development of sterile green tissue in the sporangiophore, such as sometimes occurs in *Helminthostachys*. The converse is not tenable unless we accept the view that the sterile leaf is an older structure than the sporangiophore, a view which we believe is not warranted by the facts. It has been suggested in a previous chapter that the sporophyll of the Marattiaceæ might be accounted for by a theory of concrescence of the sporangiophore and sterile lamina in the Ophioglossaceæ. *Ophioglossum palmatum*, with the two series of small sporangiophores and broad leaf lamina, is the form which comes nearest to such a condition. A very serious objection to this view was mentioned, however — the fact that the sporangiophore is an adaxial structure, whereas the synangia of the Marattiaceæ are always abaxial in their position.

Comparing the eusporangiate ferns with the leptosporangiate, it is generally conceded that the Osmundaceæ are the ferns which most closely approach the Eusporangiatæ. The Osmundaceæ show points of resemblance to both the Ophioglossaceæ and Marattiaceæ, this being true both of the vegetative tissues and the sporangia. In *Osmunda* the arrangement of the sporangia suggests *Botrychium*, while in *Todea* the sporangia are borne upon the backs of the leaves, much as they are in the Marattiaceæ. Bower has suggested that the Gleicheniaceæ, which are, however, certainly allied to the Osmundaceæ also, show certain suggestions of a marattiaceous affinity, especially in the arrangement of the sporangia.

We may then briefly summarize our conclusion as follows: From some form, allied to the simpler existing species of *Ophioglossum*, the whole fern series is descended. In this whole series the leaf is the predominant organ, the stem at first being quite subordinate in importance. This ancestral fern was monophyllous and the leaf at first was a sporophyll, perhaps without any definite sterile segment.

From this central type presumably several lines diverged, of which only a few fragments persist. One of these is seen in the different forms of *Botrychium*, to which *Helminthostachys* is probably not very remotely allied. The whole of this series is characterized by a subterranean gametophyte, and a more or less saprophytic habit of the sporophyte is indicated by the development of a mycorrhiza in the roots.

The Marattiaceæ, as they now exist, probably do not represent a single unbroken line of descent, and show strong evidences of a possible multiple derivation from the earlier stock. The point of contact with the Ophioglossaceæ is probably in the neighborhood of *Helminthostachys*, which, on the whole, is more like the Marattiaceæ than are the other Ophioglossaceæ; but it is improbable that the solid synangium, such as characterizes most of the Marattiaceæ, was derived from a group of separated sporangia like those in *Botrychium* or *Helminthostachys*, and it is more likely that they arose from a structure which resembled the spike of *Ophioglossum*. *Angiopteris* is, with little question, the most specialized of the living Marattiaceæ and has probably departed further from the ancestral type than any of the other forms, while *Kaulfussia*, on the other hand, is probably the most primitive. On the whole the Marattiaceæ are nearer to the leptosporangiate ferns than the Ophioglossaceæ are, and it is likely that the Leptosporangiates are directly descended from some ancient fern forms, allied to the Marattiaceæ, but differing from any of the existing types.

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    44. Nearly ripe spermatozoids of *Angiopteris*. × 950. *n*, nucleus; *bl*, blepharoplast; *c*, cilia; *v*, vesicle.
    45. Nearly ripe archegonium of *Danæa*, showing apparent ventral canal cell, *v*.
    46–48. Recently fertilized egg-cells of *Danæa*. *sp*, spermatozoid (?) within the egg-nucleus.

PLATE 3.

    *Ophioglossum moluccanum* Schlecht.
        Several types collected at Buitenzorg, Java, and referred to *O. moluccanum* Schlecht. There are at least three species. Fig. 5 is the typical *O. moluccanum*. All figures about natural size.

PLATE 4.

    A. Six plants of *Ophioglossum (Ophioderma) intermedium* Hk. reduced one-half. Collected at Buitenzorg, Java.
    B. *Ophioglossum (Ophioderma) pendulum* L., collected in the Hanwella Reserve Forest, Ceylon.
        1, 2. Full-grown sporophytes of *O. pendulum*, much reduced. In 2 the sporangial spike, *sp*, is forked.
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PLATE 5.

    *Ophioglossum (Cheiroglossa) palmatum* L., collected in Jamaica.
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B. Full-grown plant of *Helminthostachys*, reduced about two-thirds. Collected in the Hanwella Reserve Forest, Ceylon.

PLATE 9.

A. 1, small plant of *Danæa jenmani* Underwood; 2, rhizome of the same species; 3, apex of sterile leaf; 4, fertile leaf; 5, sterile leaf of *D. jamaicensis* Underwood.

B. 1, young plant of *Danæa elliptica* Sm. (?); 2, sporophyll of *D. jamaicensis*; 3, 4, very young plants of *D. jamaicensis* (?).
   All specimens collected in Jamaica.

PLATE 10.

Adult sporophyte of *Danæa elliptica*. Jamaica.

PLATE 11.

*Kaulfussia æsculifolia* Bl.
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PLATE 12.

A. *Marattia alata* Swz.
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   2. A single fertile pinna.
   3. Fallen leaf-base with adventitious buds, *k*.
B. *Marattia alata*.
   1. Leaf from young plant developed from an adventitious bud.
   2, 3. Young sporophytes arising from fertilization.
      All specimens from Jamaica.

PLATE 13.

*Angiopteris evecta* (Forst.) Hoffm.
   1–3. Specimens from Australia; 4, 5, specimens from Peradeniya, Ceylon.
   1, caudex of a small plant; 2, part of a sporophyll; 3, cross-section of the petiole of a full-grown leaf; 4, young sporophyte arising from the prothallium; 5, single leaf from a similar sporophyte.

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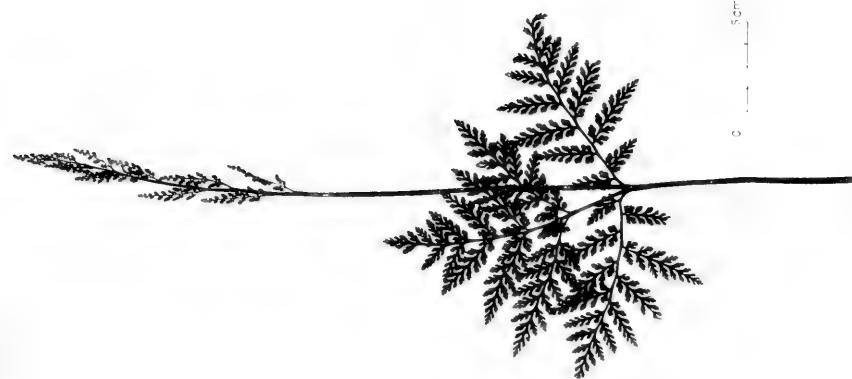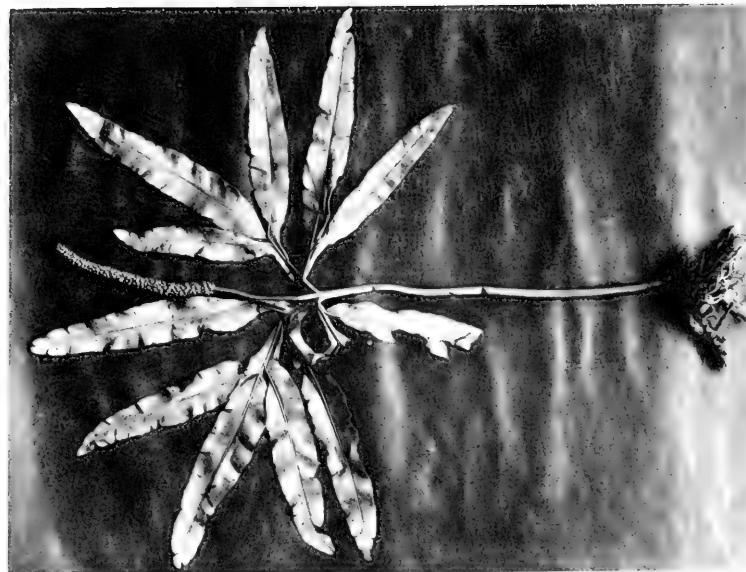


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